Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biology of the Cell 96 (2004) 327–333
Biology of the Cell
www.elsevier.com/locate/biocell

Review

Structural determinants involved in the activation and regulation of G protein-coupled receptors: lessons from the alpha1-adrenegic receptor subtypes

Susanna Cotecchia [a,*], Laura Stanasila [a], Dario Diviani [a], Katja Björklöf [a], Olivier Rossier, Francesca Fanelli [b]

[a] *Institute of Pharmacology and Toxicology, University of Lausanne, Switzerland*
[b] *Dulbecco Telethon Institute and Department of Chemistry, University of Modena and Reggio Emilia, Italy*

Received 18 December 2003; accepted 11 March 2004

Available online 11 May 2004

Abstract

The aim of a large number of studies on G protein-coupled receptors was centered on understanding the structural basis of their main functional properties. Here, we will briefly review the results obtained on the α1-adrenergic receptor subtypes belonging to the rhodopsin-like family of receptors. These findings contribute, on the one hand, to further understand the molecular basis of adrenergic transmission and, on the other, to provide some generalities on the structure-functional relationship of G protein-coupled receptors.

Keywords: Adrenergic receptors; G protein-coupled receptors; Activation; Desensitization; Oligomerization

1. Introduction

G protein-coupled receptors (GPCR), the largest group of membrane-bound receptors, are the target in the human body for a large percentage of clinically used drugs (30% of the top 50 sellers in 2001). All GPCR sequences share the presence of seven hydrophobic regions forming a bundle of transmembrane α-helices connected by alternating intracellular (i) and extracellular (e) hydrophilic loops (Fig. 1). The mammalian GPCRs can be divided in three main classes according to sequence homology: class I or rhodopsin-like (which is the largest subfamily), class II or secretin-like, and class III or glutamate-metabotropic-like. Within class I, the adrenergic receptors (AR) mediate the functional effects of epinephrine and norepinephrine. The AR family includes nine different gene products: three β (β1, β2, β3), three α2 (α2A, α2B, α2C) and three α1 (α1a, α1b, α1d) receptor subtypes.

GPCRs share three main ″classical″ functional properties: 1) they discriminate and bind the appropriate ligands; 2) they activate specific G protein-effector systems; 3) their functional response can be dynamically regulated resulting in the attenuation of receptor-mediated effects (desensitization). Beyond these classical features, a number of additional functional paradigms of GPCRs have recently emerged including constitutive activity, oligomerization and their interaction with a variety of signaling proteins.

The focus of much investigation has centered on understanding the structural basis for each of these functional properties. We have performed an extensive mutational analysis of the α1-AR subtypes aiming, on the one hand, at elucidating the molecular basis of adrenergic transmission and, on the other, at providing some generalities on the structure-functional relationship of GPCRs belonging to the rhodopsin-like subfamily.

2. Molecular modelling of the α1-AR subtypes

To probe the structure-functional relationships of GPCRs several useful molecular models of these membrane proteins were built using different methods. To investigate the potential intramolecular motions underlying different functional states of the α1-AR subtypes, we combined 3-D model build-

* Corresponding author: Prof. Susanna Cotecchia Institut de Pharmacologie et de Toxicologie Rue du Bugnon 27 1005 Lausanne, Switzerland Tel. +41-21-692 5400 Fax+ 41-21-692 5355
E-mail address: susanna.cotecchia@ipharm.unil.ch (S. Cotecchia).

doi:10.1016/j.biolcel.2004.03.009

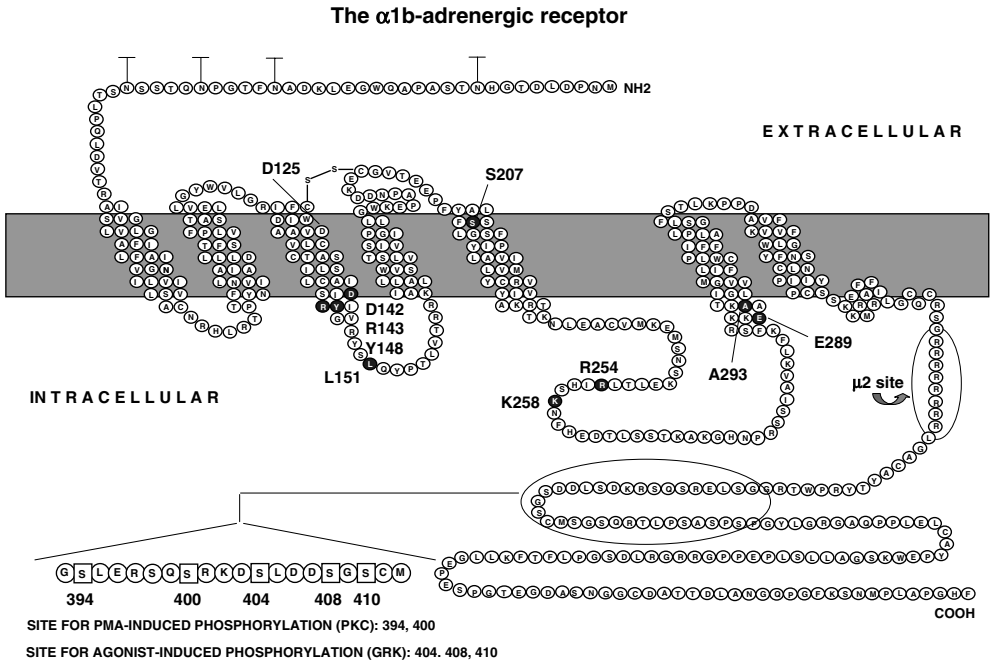

Fig. 1. Topographical model of the α1b-AR
The sequence of the hamster α1b-AR is topographically arranged according to its alignment with the crystal structure of bovine rhodopsin. The four N-linked gycosylation sites of the receptor are indicated with crosses. Key amino acids playing a role in catecholamine binding (D125, S207), receptor activation (D142, R143, E289, A293), coupling to Gq (L151, R254, K258), phosphorylation and interaction with the μ2 subunit of the AP2 complex are highlighted.

ing of the receptor structure with computational simulation of receptor dynamics (Fanelli et al., 1998). The first α1-AR model was built using an iterative *ab initio* procedure started with a comparative molecular dynamics (MD) study on the α-helical bundle of seven GPCRs (α1b-AR, α2-AR, β2-AR, dopamine D2, serotonin 5-HT1A, muscarinic M1 and the bovine rhodopsin not complexed with retinal). The arrangement of the helices was based on the structural constraints inferred from the analysis of a large number of GPCR sequences (Baldwin et al., 1997). In addition, the model was completed by adding the intracellular and extracellular loops (Fanelli et al., 1998). Recently, another model of the α1b-AR was built by comparative modeling using the recently determined 2.8Å x-ray structure of rhodopsin (Palczewski et al., 2000) as a template (Fig. 2). The analysis of the MD trajectories was used to compare the structural/dynamic features of functionally different receptor mutants with those of the wild type α1-AR subtypes, to predict key residues the mutations of which would either activate or inactivate the receptor and to investigate the potential effects of mutations (Greasley et al., 2002).

3. Mutational analysis of the ″classical″ functional properties of the α1-AR subtypes

Mutational analysis of several GPCRs has revealed that the α-helical bundle and the extracellular portions contribute to the formation of the ligand binding site, whereas the amino acid sequences of the intracellular loops appear to be mediate the interaction of the receptor with G proteins as well as with a variety of signalling and regulatory proteins (Wess, 1997).

Ligand binding. Most GPCRs undergo N-linked glycosylation. Recently it was shown that the V2 vasopressin receptor, the δ-opioid receptor and octopus rhodopsin can undergo O-linked glycosylation next to N-linked glycosylation (Petaja-Repo et al., 2000; Nakagawa et al., 2001). The role of N-glycosylation has been studied in a number of GPCRs. For some receptors it has been shown that N-linked glycosylation is important for the expression at the plasma membrane (Rands et al., 1990). However, only in few cases removal of this modification impaired receptor function. The results from our recent study on the α1b-AR, in which the four N-linked glycosylation sites were all mutated individually or in different combinations, strongly suggest that all four sites can undergo N-linked glycosylation (Fig. 1). However, mutations of the N-linked glycosylation sites did not have a significant effect on the pharmacological and signaling properties of the α1b-AR or on its expression at the cell surface (Björklöf et al., 2002). No evidence of O-linked glycosylation was found for the α1b-AR.

The molecular interactions of the endogenous catecholamines, epinephrine and norepinephrine, with different AR subtypes has been explored in different studies. Epinephrine and norepinephrine contain a protonated amino group separated from the aromatic catechol ring by a β-hydroxylethyl chain. Mutagenesis studies of the β2, α2A-AR as well as α1b-AR (Cavalli et al., 1996; Hwa et al., 1995and references herein included) suggested that the amino group of the catecholamines makes an electrostatic interaction with the carboxylate side chain of an aspartate on helix III which is highly conserved in all GPCRs binding amine ligands (Fig. 1). On the other hand, there is evidence that the catechol

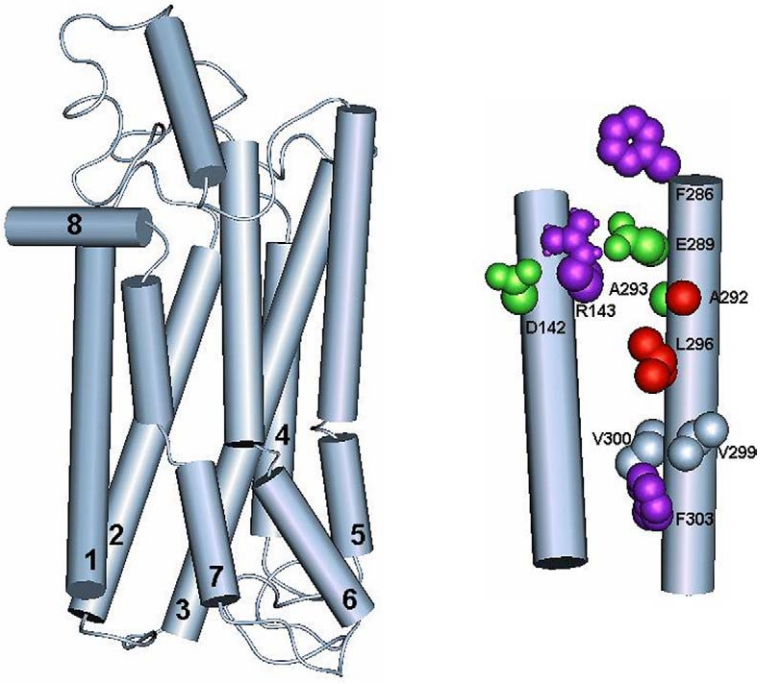

Fig. 2. Homology model of the wild type α1b-AR
Comparative modeling and Molecular Dynamic simulations were performed as described in Greasley et al., Mol Pharm. 2002. The receptor is seen from a direction parallel to the membrane surface with the extracellular side on the bottom and the intracellular one on the top. The right view displays the amino acids of helices III and VI involved in receptor activation. Van der Waals spheres whose radius has been reduced by 40% depict each side chain. The effect of mutations at each residue is depicted by their colour, with white representing no effect, green being constitutively active, red impaired receptor mediated signalling and violet being either impairing or constitutively activating depending upon the substituent amino acid.

meta-and para-hydroxyl groups interact with serine residues present in helix V of all GPCRs which bind catecholamines with high affinity (Fig. 1). In contrast, very little is known so far about the receptor amino acids which interact with different antagonists as well as about the structural basis underlying receptor selectivity for different ligands.

Receptor-G protein coupling. Several studies aimed at identifying the amino acids of different GPCRs involved in G protein coupling at both experimental and theoretical levels (Wess, 1997). However, what has become abundantly clear is that there is no simple sequence determinant that can be attributed to receptor–G protein coupling. In a previous study on chimeric β2/α1b-AR, we reported that a stretch of 27 residues of the α1b-AR (residues 233–259) derived from the N-terminal portion of its i3 loop was sufficient to confer to the β2-AR the ability to activate the Gq/PLC signaling pathway (Cotecchia et al., 1990). This provided strong evidence that the i3 loop contains the main structural determinants involved in α1b-AR coupling to Gq.

A more detailed analysis of the molecular basis of the receptor–Gq coupling was carried on recently combining molecular modeling and experimental mutagenesis of α1b-AR (Fanelli et al., 1999). Docking simulations between the α1b-AR and Gq heterotrimer led us to suggest that the positive surface of the cytosolic portion of GPCRs could complement a negative surface found on different G protein α-subunits and thereby play a role in receptor–G protein coupling. In particular, docking solutions between active forms of the α1b-AR and the Gq heterotrimer identified a number of cationic residues (R148, R160, R232, R243, R254, K258, K282, and R288) on the cytosolic surface of the receptor as being available to make contact with anionic amino acids in the αq subunit. Thus, to investigate the role of cationic residues in receptor–G protein coupling, we have mutated all the basic amino acids located in the i1, i2, and i3 loops of the α1b-AR and investigated the effect of these mutations on receptor mediated production of inositol phosphate. In addition, we have also characterised the effects resulting from mutations of conserved hydrophobic residues in the cytosolic portion of the receptor.

Among the 23 basic amino acids mutated, we found that only mutations of R254 and K258 in the i3 loop (Fig. 1) and of K291 at the cytosolic extension of helix VI markedly impaired the receptor mediated inositol phosphate production (Greasley et al., 2001). The functional analysis of the receptor mutants in conjunction with the predictions of molecular modelling support the hypothesis that R254 and K258 in the 3i loop as well as L151 in the 2i loop (Fig. 1) are directly involved in receptor-G protein interaction and/or receptor-mediated activation of the G protein. It is important to highlight that mutations of the homologous leucine or hydrophobic residue in the 2i loop resulted in receptor-G protein uncoupling for other GPCRs as well (Wess, 1997).

Receptor desensitization. Agonist-induced desensitization has been described for a variety of G protein coupled receptors (Ferguson et al., 2001). We have provided evidence

that the response mediated by the α1b-AR expressed in various cell types could undergo desensitization upon exposure to agonists as well as to the phorbol ester PMA (Lattion et al., 1994; Diviani et al., 1996). In addition, a correlation between agonist-induced phosphorylation and desensitization could be demonstrated. Our results indicated that the biochemical mechanisms underlying epinephrine versus phorbol ester-induced phosphorylation of the α1b-AR are different. This was demonstrated by the fact that the PKC inhibitor RO-318220 could abolish the effect of PMA on phosphorylation of the α1b-AR without altering that of epinephrine. Rapid agonist-dependent regulation of the α1b-AR seems, instead, to be mainly mediated by G protein-coupled receptor kinases (GRK) (Diviani et al., 1996). GRK have the unique property of phosphorylating G protein-coupled receptors once they are occupied by agonists. We demonstrated that ovexpression of GRK2 or GRK3 could increase epinephrine-induced phosphorylation of the wild type α1b-AR above basal as compared to that of the receptor expressed alone. In agreement with these findings, overexpression of the dominant negative GRK2 (K220R) mutant impaired agonist-induced phosphorylation of the receptor. Recently, we have assessed that a stretch of serines in the C-tail of the receptor represent the main sites of phosphorylation (Fig. 1). Following extensive mutagenesis studies, we have been able to identify the three serines (S404, S408 and S410) involved in agonist-induced phosphorylation from the two serines (S394 and S400) involved in PKC-mediated phosphorylation of the α1b-AR (Diviani et al., 1997). Our findings provide strong evidence that GRK2-mediated phosphorylation of Ser404, Ser408 and Ser410 is crucially involved in the desensitization of the α1b-AR. In fact, the combined mutation of these three serines impaired the receptor's ability to undergo GRK2-mediated receptor desensitization. The potential role of these serines in receptor endocytosis remains to be explored.

The findings on the α1b-AR are coherent with those reported on several other GPCRs coupled to different signalling pathways. For those receptors having a large C-tail, this receptor domain contains the main phosphorylation sites whereas phosphorylation occurs on specific sites in the large 3i loop for those GPCRs having a short C-tail.

4. Constitutively active mutants as a tool to investigate receptor activation

Agonist binding to a GPCR is believed to induce a conformational change of the receptor which results in its productive coupling to heterotrimeric G proteins thus leading to intracellular signaling events. However, a structural description of the molecular changes underlying the conversion from the inactive states (R) to the active states (R*) of the receptor is still lacking. An important contribution to our understanding of receptor activation has been provided by the finding that point mutations in the α1b-AR could increase its constitutive or agonist-independent activity (Kjelsberg et al., 1992). These findings suggested that in the absence of agonist a structural constraint keeps the wild type receptor inactive (R) preventing sequences of the intracellular loops to interact with the G proteins. Activating mutations might release such constraint triggering the conversion into the active state (R*), which couples to G proteins. One hypothesis is that activating mutations mimic, at least to some extent, the conformational change triggered by agonist binding to GPCR. This hypothesis was supported by a detailed analysis of the properties of the AR constitutively active mutants (CAMs) which proposed the ″allosteric ternary complex model″ (Samama et al., 1993). This extended version of the ternary complex model introduced for the first time an explicit isomerization constant regulating the equilibrium of GPCR between at least two interconvertible allosteric states, R (inactive or ground state) and R* (active).

Which is the nature of the ″constraint″? is it similar for GPCRs of different classes? how can mutations in apparently unrelated regions of a GPCR release this constraint? Are CAMs representative of the agonist-bound wild type receptor? A large number of studies have addressed these questions and the results from some of them are summarized here below (reviewed in Gether, 2000).

Studies from our laboratory combining site-directed mutagenesis of the α1b-AR and molecular dynamics simulations of receptor models highlighted the important role played in receptor activation by the E/DRY motif at the cytosolic end of helix III (Fig. 1), which is highly conserved in GPCRs of the rhodpsin-like class. All possible amino acid substitutions of the aspartate of the E/DRY sequence increased the constitutive activity of the α1b-AR at different extent (Scheer et al., 1997). Increased constitutive activity was also found after mutating the acidic residue of the E/DRY motif in other receptors including rhodopsin (Cohen et al., 1993) and the β2-AR (Rasmussen et al., 1999). In contrast, mutations of the conserved arginine in a number of GPCRs can profoundly impair receptor function (Wess, 1997, Scheer et al., 2000).

Different mechanisms have been proposed to explain the role of the E/DRY motif in GPCR activation. In particular, we highlighted that the interactions between the arginine of the E/DRY and some amino acids forming a highly conserved ″polar pocket″ within the helical bundle contribute to stabilize the ground state of the α1b-AR (Scheer et al., 1996). The recently published structure of rhodopsin in its inactive state suggests that the arginine of E/DRY sequence interacts with both the adjacent glutamic acid and a glutamate on helix VI ($E247^{6.30}$) (Palczewski et al., 2000). Thus, in rhodopsin both the inter-helical and intra-helical salt bridges involving the highly conserved arginine of the E/DRY motif of helix III might play a role in stabilizing the dark state. Similarly to the rhodopsin structure, in the majority of the average minimized structures of the wild type α1b-AR obtained following MD simulations on a rhodopsin-based (homology) model, the arginine of the E/DRY motif makes a salt bridge with both

the adjacent aspartate and a glutamate (E289$^{6.30}$) (Fig. 2). Similarly to the effect induced by mutating the D142$^{3.49}$ of the E/DRY motif, mutations of the E289$^{6.30}$ markedly increased the constitutive activity of the α1b-AR (Greasley et al., 2002). Therefore, whereas the interaction of the arginine with E289$^{6.30}$ constrains the receptor in the inactive state, its breakage would contribute to receptor activation. The hypothesis that the motion of the conserved arginine is constrained by both D142$^{3.49}$ and E289$^{6.30}$ is supported by the results of MD simulations on a large number of α1b-AR mutants, including D142A and E289K. Indeed, the structures of the active mutants are characterized by the release or weakening of both the charge reinforced H-bonding interactions involving R143$^{3.50}$ in the wild type or inactive state (Greasley et al., 2002). The structural conservation among different receptors suggests that the constraining interactions involving the E/DRY sequence might be a common feature of GPCRs belonging to the rhodopsin-like class (reviewed in Gether, 2000; Parnot et al., 2002). However, for GPCRs of the secretin-and metabotropic glutamate-like classes, lacking the E/DRY motif, the mechanisms of activation might involve other structural determinants of the receptor. In addition, other potentially constraining interactions have been identified in GPCRs beyond those involving helices III and VI (reviewed in Parnot et al., 2002). Altogether, these findings indicate that the analysis of CAMs has been instrumental to generate hypothesis on the molecular basis of receptor activation. However, because of the absence of structural information on GPCRs, a detailed mechanism describing receptor activation is still lacking. A particularly important challenge for the future will be to explain how agonists can activate GPCRs and to what extent a common mechanism of activation is shared by GPCRs of different classes.

5. Novel protein interactions involved in receptor regulation

Recently it is has become increasingly apparent that, in addition to signalling via G proteins, GPCRs can act as scaffolds binding a variety of proteins and this might promote the activation of novel G protein-independent signalling pathways (reviewed in Pierce et al., 2002). In fact a number of proteins have been found to interact with GPCRs using the yeast-2-hybrid system and other biochemical methods. Beyond GRKs, βarrestins and heterotrimeric G proteins subunits, the list of proteins interacting with GPCRs include: calmodulin, calcyon, AKAP, RAMPs (receptor-associated modulating proteins), tubulin, SH3 domain-containing adaptor molecules like Grb2 and Src, EBP50 (Ezrin binding phosphoprotein 50)/NHERF (Na^+/H^+ exchanger-regulatory factor) and PSD95 (post-synaptic density 95). The functional implications of these interactions are not fully understood; however they add an increasing complexity to the signalling mechanisms mediated by GPCRs.

To identify new proteins interacting with the α1b-AR that could potentially be involved in regulating receptor function, we used the C-tail of the receptor as a bait in the yeast two-hybrid system and identified the μ2 subunit of the AP2 complex as a binding partner. The AP2 complex is known to directly link the clathrin coat with cargo transmembrane proteins that are sorted into coated pits and vesicles (Haucke et al., 2000) and is composed of two large subunits, α and β2 of about 100 kDa, and two smaller subunits, μ2 and α2 of 50 and 17 kDa, respectively. The AP2 adaptor can initiate endocytosis of single membrane spanning domain receptors by associating directly with their cytoplasmic tail (Pearse et al., 2000). Direct association between the C-tail of the α1b-AR and μ2 was demonstrated using a solid phase overlay assay (Diviani et al., 2003). The α1b-AR/μ2 interaction occurred inside the cells as shown by the finding that the transfected α1b-AR and the endogenous μ2 could be coimmunoprecipitated from HEK-293 cell extracts. Mutational analysis of the α1b-AR revealed that the binding site for μ2 does not involve canonical YXXΦ or dileucine motifs, but a stretch of eight arginines on the receptor C-tail (Fig. 1). The binding domain of μ2 for the receptor C-tail involves both its N-terminus and the subdomain B of its C-terminal portion. The α1b-AR specifically interacted with μ2, but not with the μ1, μ3 or μ4 subunits belonging to other AP complexes. The deletion of the μ2 binding site in the C-tail markedly decreased agonist-induced receptor internalization as demonstrated by confocal microscopy as well as by the results of a surface receptor biotinylation assay.

The direct association of the AP2 complex with a GPCR might represent a common mechanism underlying clathrin-mediated receptor endocytosis. Previous studies have shown that the AP2 complex is implicated in the agonist-induced endocytosis of the β2-AR (Laporte et al., 1999; Kim et al., 2002). However, in this case the AP2 complex seemed to be recruited to the receptor through β-arrestins which can directly interact with the μ2 subunit of AP2 (Pearse et al., 2000). Our findings on the α1b-AR indicate that, in addition to β-arrestins, the direct association of the receptor with the AP2 complex plays an important role in the clathrin-mediated endocytosis of GPCRs. Future studies should aim at elucidating the relationship between the structural determinants of GPCRs involved in binding the AP2 complex versus β-arrestins, the respective role of the AP2 complex and β-arrestins in targeting the receptor to clathrin-coated vesicles as well as their interplay with other yet unidentified mechanisms regulating receptor trafficking and function.

6. Receptor oligomerization

GPCRs were for a long time presumed to function as monomers according to the prevailing model: one ligand molecule-one receptor-one G protein (Angers et al., 2002). Recently, increasing complexity of GPCR function and regulation has progressively emerged. For example, one GPCR can adopt multiple conformational states able to differentially interact with signaling and regulatory proteins (Gha-

nouni et al., 2001). In addition, it was shown that cross-talk among GPCRs can also occur at the receptor level by means of receptor oligomerization (reviewed in Gomes et al., 2001). We combined biophysical, biochemical and pharmacological approaches to investigate the ability of the α1a and α1b-AR subtypes to form homo- and hetero-oligomers (Stanasila et al., 2003). Receptors tagged with different epitopes (HA and myc) or fluorescent proteins (CFP and GFP) were transiently expressed in HEK-293 cells either individually or in different combinations. Fluorescence resonance energy transfer (FRET) measurements provided evidence that both the α1a and α1b-AR can form homo-oligomers with similar transfer efficiency of ~0.10. Hetero-oligomers could also be observed between the α1b-and the α1a-AR subtypes, but not between the α1b-AR and the β2-AR, the NK1 tachykinin or the CCR5 chemokine receptors. Oligomerization of the α1b-AR did not require the integrity of its C-tail, of two glycophorin motifs or of the N-linked glycosylation sites at its N-terminus. In contrast, helix I and, to a lesser extent, helix VII were found to play a role in the α1b-AR homo-oligomerization. Receptor oligomerization was not influenced by the agonist epinephrine or by the inverse agonist prazosin. A constitutively active (A293E) as well as a signaling-deficient (R143E) mutant displayed oligomerization features similar to those of the wild type α1b-AR.

Confocal imaging revealed that oligomerization of the α1-AR subtypes correlated with their ability to co-internalize upon exposure to the agonist. The α1a-selective agonist oxymetazoline induced the co-internalization of the α1a- and α1b-AR, whereas the α1b-AR could not co-internalize with the NK1 tachykinin or CCR5 chemokine receptors. Oligomerization might therefore represent an additional mechanism regulating the physiological responses mediated by the α1a and α1b-AR subtypes. The two receptors have an overlapping distribution in several tissues (e.g. heart, brain, prostate, etc.). One can expect that hetero-oligomerization might provide yet another means to fine tune the responses mediated by the α1a and α1b-AR subtypes like, for example, coordinating their internalization properties. GPCR oligomerization has clearly emerged as an important functional paradigm. It is possible that, despite some common features, the oligomerization mechanisms might differ among GPCRs thus increasing the complexity of receptor signaling and regulation. Future studies should aim at investigating the role of receptor oligomerization in physiological systems as well as at further unraveling its molecular basis.

7. Conclusions

Our mutational analysis on the α1-AR subtypes has clearly provided some generalities on the structure-functional relationship of GPCRs belonging to the rhodopsin-like family of receptors. However, structural information at high resolution on other GPCRs than rhodopsin will be necessary to challenge the predictions of molecular modeling and improve our understanding of GPCR activation and drug action at a molecular level.

Acknowledgements

The work in S. Cotecchia's laboratory was supported by the Fonds National Suisse de la Recherche Scientifique (grant n. 31000A0-10073). F. Fanelli is supported by the Telethon-Italy grant n. TCP00068.

References

Angers, S., Salahpour, A., Bouvier, M., 2002. Dimerization: an emerging concept for G protein-coupled receptor ontogeny and function. Annu Rev Pharmacol Toxicol 42, 409–435.

Baldwin, J.M., Schertler, G.F., Unger, V.M., 1997. An Alpha-Carbon Template for the Transmembrane Helices in the Rhodopsin Family of G Protein-Coupled Receptors. J. Mol. Biol. 272, 144–164.

Björklöf, K., Lundstrom, K., Abuin, L., Greasley, P.J., Cotecchia, S., 2002. Coand posttranslational modification of the alpha(1B)-adrenergic receptor: effects on receptor expression and function. Biochemistry 41, 4281–4291.

Cavalli, A., Fanelli, F., Taddei, C., De Benedetti, P.G., Cotecchia, S., 1996. Amino Acids of the Alpha1B-Adrenergic Receptor Involved in Agonist Binding: Differences in Docking Catecholamines to Receptor Subtypes. FEBS Lett. 399, 9–13.

Cohen, G.B., Yang, T., Robinson, P.R., Oprian, D.D., 1993. Constitutive activation of opsin: influence of charge at position 134 and size at position 296. Biochemistry 32, 6111–6115.

Cotecchia, S., Exum, S., Caron, M.G., Lefkowitz, R.J., 1990. Regions of α1-adrenergic receptor involved in coupling to phosphatidylinositol hydrolysis and enhanced sensivity of biological function. Proc Natl Acad Sci USA 87, 2896–2900.

Diviani, D., Lattion, A.L., Larbi, N., Kunapuli, P., Pronin, A., Benovic, J.L., Cotecchia, S., 1996. Effect of different G protein-coupled receptor kinase- and protein kinase C-mediated desensitization of the alpha1B-adrenergic receptor. J Biol Chem 271, 5049–5058.

Diviani, D., Lattion, A.L., Cotecchia, S., 1997. Characterization of the phosphorylation sites involved in G protein-coupled receptor kinaseand protein kinase C-mediated desensitization of the alpha1Badrenergic receptor. J Biol Chem 272, 28712–28719.

Diviani, D., Lattion, A.L., Abuin, L., Staub, O., Cotecchia, S., 2003. The adaptor complex 2 directly interacts with the alpha1b-adrenergic receptor and plays a role in receptor endocytosis. J Biol Chem 278, 19331–19340.

Fanelli, F., Menziani, C., Scheer, A., Cotecchia, S., De Benedetti, P.G., 1998. Ab initio Modeling and Molecular Dynamics Simulation of the Alpha1bAdrenergic Receptor Activation. Methods 14, 302–317.

Fanelli, F., Menziani, C., Scheer, A., Cotecchia, S., De Benedetti, P.G., 1999. Theoretical Study on the Electrostatically Driven Step of Receptor–G Protein Recognition. Proteins: Structure, Function and Genetics 37, 145–156.

Ferguson, S.S., 2001. Evolving concepts in G protein-coupled receptor endocytosis: the role in receptor desensitization and signaling. Pharmacol Rev 53, 1–24.

Gether, U., 2000. Uncovering molecular mechanisms involved in activation of G protein-coupled receptors. Endocr Rev 21, 90–113.

Ghanouni, P., Gryczynski, Z., Steenhuis, J.J., Lee, T.W., Farrens, D.L., Lakowicz, J.R., Kobilka, B.K., 2001. Functionally different agonists induce distinct conformations in the G protein coupling domain of the beta2 adrenergic receptor. J Biol Chem 276, 24433–24436.

Gomes, I., Jordan, B.A., Gupta, A., Rios, C., Trapaidze, N., Devi, L.A., 2001. G protein coupled receptor dimerization: implications in modulating receptor function. J Mol Med 79, 226–242.

Greasley, P., Fanelli, F., Scheer, A., Abuin, L., Nenniger-Tosato, M., De Benedetti, P.G., Cotecchia, S., 2001. Mutational and computational analysis of the alpha1b-adrenergic receptor: involvement of basic and hydrophobic residues in receptor activation and G protein coupling. J Biol Chem 276, 46485–46494.

Greasley, P., Fanelli, F., Rossier, O., Abuin, L., Cotecchia, S., 2002. Mutagenesis and modelling of the alpha1b-adrenergic receptor highlights the role of the helix3/helix 6 interface in receptor activation. Mol Pharmacol 61, 1025–1032.

Haucke, V., Wenk, M.R., Chapman, E.R., Farsad, K., De Camilli, P., 2000. Dual interaction of synaptotagmin with mu-2 and alpha-adaptin facilitates clathrin-coated pit nucleation. EMBO J 19 (22), 6011–6019.

Hwa, J., Graham, R.M., Perez, D.M., 1995. Identification of Critical Determinants of Alpha1-Adrenergic Receptor Subtype Selective Agonist Binding. J Biol Chem 270, 23189–23195.

Kim, Y.M., Benovic, J.L., 2002. Differential roles of arrestin-2 interaction with clathrin and adaptor protein 2 in G protein-coupled receptor trafficking. J Biol Chem 277, 30760–30768.

Kjelsberg, M.A., Cotecchia, S., Ostrowski, J., Caron, M.G., Lefkowitz, R.J., 1992. Constitutive activation of the alpha1B-adrenergic receptor by all amino acid substitution at a single site. J Biol Chem 267, 1430–1433.

Laporte, S.A., Oakley, R.H., Zhang, J., Holt, J.A., Ferguson, S.S., Caron, M.G., Barak, L.S., 1999. The beta2-adrenergic receptor/beta-arrestin complex recruits the clathrin adaptor AP-2 during endocytosis. Proc Natl Acad Sci U S A 96, 3712–3717.

Lattion, A.L., Diviani, D., Cotecchia, S., 1994. Truncation of the receptor impairs agonist-induced phosphorylation and desensitization of the alpha1B-adrenergic receptor. J Biol Chem 269, 22887–22893.

Nakagawa, M., Miyamoto, T., Kusakabe, R., Takasaki, S., Takao, T., Shichida, Y., Tsuda, M., 2001. O-glycosylation of G-protein-coupled receptor, octopus rhodopsin. Direct analysis by FAB mass spectrometry. FEBS Lett 496 (1), 19 24.

Palczewski, K., Kumasaka, T., Hori, T., Behnke, C.A., Motoshima, H., Fox, B.A., Le Trong, I., Teller, D.C., Okada, T., Stenkamp, R.E., Yamamoto, M., Miyano, M., 2000. Crystal structure of rhodopsin: A G protein-coupled receptor. Science 289, 739–745.

Parnot, C., Miserey-Lenkei, S., Bardin, S., Corvol, P., Clauser, E., 2002. Lessons from constitutively active mutants of G protein-coupled receptors. Trends Endocrinol & Metabol 13, 336–343.

Pearse, B.M., Smith, C.J., Owen, D.J., 2000. Clathrin coat construction in endocytosis. Curr Opin Struct Biol 10, 220–228.

Petaja-Repo, U.E., Hogue, M., Laperriere, A., Walker, P., Bouvier, M., 2000. Export from the endoplasmic reticulum represents the limiting step in the maturation and cell surface expression of the human delta opioid receptor. J Biol Chem 275 (18), 13727–13736.

Pierce, K.L., Premont, R.T., Lefkowitz, R.J., 2002. Signalling: Seventransmembrane receptors. Nat Rev Mol Cell Biol 3, 639–650.

Rands, E., Candelore, M.R., Cheung, A.H., Hill, W.S., Strader, C.D., Dixon, R.A., 1990. Mutational analysis of beta-adrenergic receptor glycosylation. J Biol Chem 265 (18), 10759–10764.

Rasmussen, S.G.F., Jensen, A.D., Liapakis, G., Ghanouni, P., Javitch, J.A., Gether, U., 1999. Mutation of highly conserved aspartic acid in the beta2 adrenergic receptor: constitutive activation, structural instability and conformational rearrangement of transmembrane segment 6. Mol Pharmacol 56, 175–184.

Samama, P., Cotecchia, S., Costa, T., Lefkowitz, R.J., 1993. A mutation-induced activated state of the beta2-adrenergic receptor: extending the ternary complex model. J Biol Chem 268, 4625–4636.

Scheer, A., Fanelli, F., Costa, T., De Benedetti, P.G., Cotecchia, S., 1997. The activation process of the alpha1B-adrenergic receptor: potential role of protonation and hydrophobicity of a highly conserved aspartate. Proc Natl Acad Sci U S A 94, 808–813.

Scheer, A., Costa, T., Fanelli, F., De Benedetti, P.G., Mhaouty-Kodja, S., Abuin, L., Nenniger-Tosato, M., Cotecchia, S., 2000. Mutational analysis of the highly conserved arginine within the Glu/Asp-Arg-Tyr motif of the alpha(1b)-adrenergic receptor: effects on receptor isomerization and activation. Mol Pharmacol 57, 219–231.

Stanasila, L., Perez, J.B., Vogel, H., Cotecchia, S., 2003. Oligomerization of the alpha1a- and alpha1b-adrenergic receptor subtypes. Potential implications in receptor internalization. J Biol Chem 278 (41), 40239–40251.

Wess, J., 1997. G-protein-coupled receptors: molecular mechanisms involved in receptor activation and selectivity of G-protein recognition. FASEB J 5, 346–354.