Homo- and hetero-oligomerization of β2-adrenergic receptor in receptor trafficking, signaling pathways and receptor pharmacology

Author Manuscript

Artur Wnorowski, Krzysztof Jozwiak

Laboratory of Medicinal Chemistry and Neuroengineering, Department of Chemistry, Medical University of Lublin, 20-093 Lublin, Poland

Highlights

- β2-Adrenergic receptor (β2AR) forms homo- and hetero-oligomers.
- β2ARs exist predominantly as dimers.
- β2AR facilitates cell surface delivery of otherwise poorly expressed receptors.
- Heterotropic interactions modulate signaling outputs from β2AR.
- β2AR hetero-oligomers constitute very interesting target for future drugs.

Abstract

The β2-adrenergic receptor (β2AR) is the prototypic member of G protein-coupled receptors (GPCRs) involved in the production of physiological responses to adrenaline and noradrenaline. Research done in the past few years vastly demonstrated that β2AR can form homo- and hetero-oligomers. Despite the fact that currently this phenomenon is widely accepted, the spread and relevance of β2AR oligomerization are still a matter of debate. This review considers the progress achieved in the field of β2AR oligomerization with focus on the implications of the receptor–receptor interactions to β2AR trafficking, pharmacology and downstream signal transduction pathways.

Abbreviations

AR, adrenergic receptor; AT1R, angiotensin II type 1 receptor; Bk2, Rbradykinin type 2 receptor; BRET, bioluminescence resonance energy transfer; cAMP, cyclic adenosine monophosphate; CB1R, cannabinoid type 1 receptor; Co-IP, co-immunoprecipitation; CREB, cAMP response element binding protein; ER, endoplasmic reticulum; ERK1/2, extracellular-signal-regulated kinase ½; FRAP, Fluorescence recovery after photobleaching; FRET, fluorescence resonance energy transfer; GPCR, G-protein-coupled receptor; H, helix; HA, hemagglutinin; M71OR, murine 71 olfactory receptor; MAPK, mitogen-activated protein kinase; μOR, μ opioid receptor; OTR, oxytocin receptor; RET, resonance energy transfer; Rluc, Renilla luciferase; SDF-1, αstromal cell-derived factor-1; SSTR5, somatostatin receptor 5; TIRF-M, total internal reflection fluorescence microscopy; TM, transmembrane helix; T-tubule, transverse-tubular regions; YFP, yellow fluorescent protein.

Keywords

Receptor mosaic, Receptorosome, Receptor–receptor interaction, Oligomerization interface, Beta-adrenoceptor

Disclaimer

This is a slightly polished and reformatted version of the following manuscript: Wnorowski A, Jozwiak K. *Homo- and hetero-oligomerization of β2-adrenergic receptor in receptor trafficking, signaling pathways and receptor pharmacology*, which has been published in final form at https://doi.org/10.1016/j.cellsig.2014.06.016 (Cell Signal. 2014 Oct;26(10):2259-65. doi: 10.1016/j.cellsig.2014.06.016. Epub 2014 Jul 15).

1 Introduction

The β2-adrenergic receptor (β2AR) is the prototypic member of ubiquitous cell-surface proteins known as G protein-coupled receptors (GPCRs) or seven transmembrane receptors. GPCRs are capable of binding various types of ligands and thus transduce and control signals related to multiple processes like neurotransmission, metabolism, cell growth and immune response. Traditional model of GPCR signaling is based on the assumption that ligand binding and specific downstream signaling events are mediated by activity of a monomeric receptor, i.e. the ligand, receptor and G protein interact in a 1:1:1 stoichiometry. However, this model is currently deemed oversimplified. Research done in recent years has revealed that not only the activity of a particular class of G proteins is driven by dimerization [1], but also the GPCRs themselves can directly interact to form homo- and hetero-oligomers [2].

β2AR is involved in production of physiological responses to adrenaline and noradrenaline. Several distinct signaling pathways can be triggered upon activation of this receptor. β2AR has been reported to couple predominantly with stimulatory Gαs and to a lesser extent with inhibitory Gαi to modulate the activity of adenylyl cyclase and mitogen-activated protein kinases (MAPKs). Additionally, there is a growing list of proteins directly interacting with β2AR to produce physiologically relevant outcomes in a G protein-independent manner [3]. Particularly, the β-arrestin, scaffold protein involved in desensitization of the receptor, has been identified as a potent activator of MAPK cascade that does not require G protein coupling [4]. β-Arrestin may also facilitate the recruitment of additional signaling molecules to the intracellular moiety of the receptor.

Oligomerization broadens the spectrum of potential downstream signaling capabilities of β2AR even further. Outcomes of activation of oligomeric β2AR may exceed the functional characteristics of the monomeric receptor and apply to receptor trafficking, signaling pathways and receptor pharmacology. The scope of this review is to underline the implications of β2AR oligomerization and development in techniques used to study this phenomenon.

2 Homo-oligomerization

First indirect pieces of evidence for the existence of β-adrenergic receptor homodimers were obtained from pharmacological studies, where negatively cooperative site–site interactions occurred among βARs originated from different tissues [5–7]. In hindsight, this was explained by the presence of an additional ligand binding site resulting from receptor dimerization [8]. Further indications for direct interactions between the two β2ARs were acquired from immunoaffinity chromatography [9] and transmission electron microscopy [10]. Experiments involving co-immunoprecipitation (Co-IP) of differentially epitope-tagged receptors and western blot assays also pointed toward the phenomenon of β2AR dimerization [11]. Co-expressed β2AR tagged with either c-myc or hemagglutinin (HA) subjected to immunoprecipitation with anti-c-myc antibody and subsequent blotting with anti-HA antibody enabled the visualization of the receptor dimers composed of both c-myc and HA-tagged protomers [11].

Despite the growing body of biochemical and functional evidence supporting the notion that β2ARs form homodimers, doubts arose over the relevance of these findings for living cells. The main concern was that the solubilization and extraction of highly hydrophobic receptors from the membrane might lead to artifactual protein aggregation and false-positive results [12–14]. To address this issue, bioluminescence resonance energy transfer (BRET), a novel biophysical technique that was previously used to examine interactions between the bacterial circadian clock proteins [15], was utilized by Angers and colleagues [13]. BRET approach is based on nonradiative transfer of energy from luminescent donor protein to fluorescent acceptor. Generally, the energy transfer occurs when the donor and acceptor are 1 to 10 nm apart, thus allowing assessment of the proximity between the interacting molecules [16]. Constructs consisted of human β2AR fused with either Renilla luciferase (Rluc) or yellow fluorescent protein (YFP) were generated and tested for BRET occurrence at plasma membrane of cultured cells delivering unambiguous confirmation on the β2AR homodimer formation in intact cells [13].

In significance, these studies have not ruled out the possibility that β2ARs form oligomers that are consisted of more than just two protomers. Further research was to elucidate how large the functional oligomers can be and whether only one particular β2AR oligomer type exists or there is an equilibrium between monomers and different types of oligomers. Fung and colleagues employed fluorescence resonance energy transfer (FRET) technique to gain more insight into the spatial arrangement and number of β2AR protomers interacting within a single receptor complex [17]. Purified β2ARs were modified with three different small-molecular-weight fluorescent probes and embedded within a lipid bilayer. Applied mathematical model explained that the lower FRET saturative acceptor to donor ratio was the more protomers were packed together into a single oligomer. The authors anticipated that β2ARs exist predominantly as tetramers; however, binding of an inverse agonist (ICI 118,551), but not full agonist (isoproterenol) nor neutral antagonist (alprenolol), induced formation of higher-order β2AR oligomers–octamers [17]. On the other hand, introduction of Gs protein to the system led to oligomer destabilization [17]. This may form a premise supporting the notion that highly packed β2AR assemblies constitute an inactive form of the receptor.

One has to bear in mind that the described resonance energy transfer (RET) approaches rely on the exogenous overexpression of the modified receptor in immortalized cells or receptor reconstitution in artificial membranes. Therefore, the experimental concentration of β2AR may considerably exceed the expression of β2AR observed in native tissues. As a result, excessive crowding of the receptor molecules at the membrane could possibly lead to the detection of unspecific receptor clusters that do not represent functional oligomers in physiological conditions [18].

In fact, the possibility of RET occurrence due to random collisions was intensively discussed in the literature [18–21]. This debate led to employment of novel techniques to study the dynamics of oligomerization phenomenon. Fluorescence recovery after photobleaching (FRAP), an optical technique that

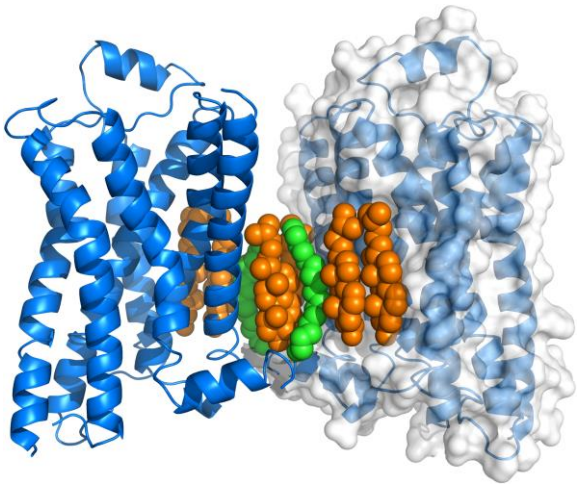

Fig. 1. Structure of human β2AR receptor homodimer. β2AR protomers are presented as blue cartoon models. The transparent surface of the molecule is shown for the protomer on the right. Majority of the dimerization interface is constituted by ordered lipids: 6 cholesterol molecules (orange) and two palmitic acid molecules (green) covalently linked to cysteine residues of the respective protomers. Rendered based on PDB: 2RH1 [24].

examines two dimensional mobility of fluorescent probes, was applied to measure the extent and stability of β2AR oligomerization in cultured cells. During the typical FRAP experiment the lateral diffusion of fluorescently-tagged receptors to intentionally photobleached region of plasma membrane is analyzed. Data obtained from this type of studies indicated the existence of stable tetra- or pentamers of composition unaffected by stimulation with agonist (isoproterenol) or antagonist (propranolol) [22]. Recent study of Calebiro and colleagues applied SNAP-tag labeling combined with total internal reflection fluorescence microscopy (TIRF-M) to track single receptor molecules at the surface of intact cells and thus monitor changes in their spatial and temporal organization [23]. Observation of individual receptors showed that β2AR existed as a mixture of monomers, dimers and higher order oligomers with the composition of receptor complexes correlated with the receptor particles concentration at the plasma membrane. At receptor density of 0.15–0.3 particle/μm2 around 60% of the β2AR population was constituted by dimers with remaining 40% being monomers. However, at higher density (0.4–0.45 particle/μm2) monomers and dimers were accompanied by tri- and tetramers. Notably, receptor densities observed in this study were comparable to the receptor concentration in some cell lines endogenously expressing β2AR [23].

In-depth analysis of receptor movement indicated that typical β2AR oligomer lifetime is about 5 s long and occurs due to combination of true receptor–receptor interactions and transient collisions, with the contribution of the later component of roughly 20%. Consequently, β2AR oligomerization appears to be based on cycles of rapid binding, interaction and disassociation of highly dynamic protomers [23].

2.1 Oligomerization interface

High-resolution crystal structure of an engineered human β2AR revealed that the dimerization interface between the two β2AR molecules is established by transmembrane helix 1 (TM1) and cytoplasmic helix 8 (H8) of each of the protomers [24]. The majority of the interactions were mediated by ordered lipids whereas direct receptor–receptor contact was limited to the cytoplasmic end of the interface where ion bounding between Lys159 and Glu338 occurred [24]. The stoichiometry of lipid–receptor dimerization interface was three cholesterol molecules and one covalently attached palmitic acid molecule per monomer (Fig. 1). Thioesterification of Cys341 in the carboxyl tail of the protein with palmitic acid is essential for β2AR activity since mutation of this residue to glycine prevented its palmitoylation and resulted in constitutive desensitization of the receptor [25]. Interestingly, dimerization between wild-type β2AR and palmitoylation-defective Cys341Gly mutant led to functional complementation of impaired phenotype and restored lost activity of the mutant [26].

The importance of TM1/H8 interface for β2AR oligomerization was also supported by FRET experiments, where the greatest energy transfer (i.e. the lowest distance) was observed for probes attached to H8 of the interacting protomers, emphasizing the significance of this domain and the adjacent TM1 for β2AR oligomerization [17]. This data is in agreement with studies demonstrating the significance of TM1 of other family A GPCRs [27,28] in the formation of homo-oligomerization interface. Recently obtained crystal structures of turkey β1AR and murine μ opioid receptor (μOR) display that both of those GPCRs form two distinct oligomerization interfaces [29,30]. The first interface engages residues from TM1, TM2 and H8, similarly to the interface observed in the crystal structure of β2AR [24]. The second interface is comprised of TM4/TM5 and TM5/TM6 for β1AR and μOR, respectively. Although, the existence of this second interaction interface is not confirmed by available crystal structures of β2AR, there is a number of functional data indicating that the second dimerization interface exists in TM6 region of the β2AR [11,31]. Hebert and colleagues demonstrated that leucine and glycine residues (underlined) within TM6: 272LKTLGIIMGTFTL284 formed a crucial element involved in the interaction between β2AR proteins [11]. A peptide derived from TM6 was able to specifically interfere with β2AR dimer formation in a time-dependent fashion [11]. Further studies indicated that 284Leu residue was crucial for dimerization process, as β2AR constructs harboring tyrosine or glycine at this position showed significantly impaired dimerization [31]. Noteworthy, multiple studies on β2AR homomerization that applied co-immunoprecipitation and BRET could not distinguish whether assembly of β2AR oligomers was mediated by direct receptor–receptor interactions or that the protomers were tethered into complexes by scaffold proteins. The experiments of Fung and colleagues utilized highly purified receptors and model lipid vesicles, thus demonstrating that β2AR oligomerization and potential ligand-induced protomer rearrangements were independent from assistance of other proteins [17].

Recently, techniques of computational simulation were employed to study the mechanism of self-assembly of β2AR [32,33]. Molecular dynamics carried out by Ghosh and colleagues demonstrated that most of the interaction surfaces established between the simulated receptors were composed of TM1/TM1, H8/H8, TM1/TM5 and TM6/TM6 [33]. This observation stays in line with experimental data and thus indicates that in silico analyses may be useful for studying GPCR oligomerization.

2.2 Receptor trafficking

Several studies elaborated on the influence of oligomerization on the β2AR trafficking. Modified β2AR containing C-terminal endoplasmic reticulum (ER)-retention motif failed to reach cell surface and prevented cell surface delivery of co-expressed wild-type β2AR [31]. Similarly, dimerization-impaired mutant was retained in the ER, thus exhibiting greatly reduced plasma membrane concentration compared to the wild-type receptor [31]. These observations strongly indicated that β2AR oligomerization originates within the ER and is essential for the receptor maturation and trafficking to the cell surface.

It is well established that once at the plasma membrane β2AR undergoes robust agonist-induced internalization to endosomes [34]. Experiments of Sartania and colleagues demonstrated that co-expression of isoproterenol-insensitive Asp113Ser β2AR mutant together with the wild-type receptor resulted in internalization of both the wild-type and the mutated β2AR after challenge with either isoproterenol or Asp113Ser mutant-specific agonist [35]. The authors concluded that agonist binding to the single protomer is sufficient to induce internalization of the whole β2AR oligomer [35]. On the contrary, another study reported that binding of agonist involves disruption of a fraction of interactions between β2ARs and may result in predominant internalization of agonist-bound protomers [36]. This stayed in line with the work of Gavalas and colleagues who suggested that the interaction between β2AR protomers was not strong enough to enable the recruitment of one receptor species by another one to a cell surface microdomain [37]. In light of these data the involvement of oligomerization in cellular distribution of β2AR may differ depending on trafficking step and cellular context.

3 Hetero-oligomerization of βARs

After the discovery that β2AR forms homo-oligomers, it was tempting to investigate whether β2AR can oligomerize with two other closely related β-adrenergic receptors, i.e. β1AR and β3AR (Table 1). All three receptors share a high degree of sequence identity, especially within transmembrane spanning domains proposed as oligomerization interface. Noteworthy, both β1AR and β3AR were shown to homo-oligomerize when expressed in heterologous expression systems [38,39]. Furthermore, β2AR and β1AR are co-expressed in cardiomyocytes whereas β2AR and β3AR can both be found in adipocytes. These facts suggest the possibility that these receptors form hetero-oligomers in a native environment.

Interactions between β1AR and β2AR in cultured cells were assessed by means of co-immunoprecipitation and BRET measurements [40–42]. The analysis revealed that β1AR and β2AR readily engaged into formation of homo- and hetero-oligomers even at expression levels comparable to those observed for dog and human heart tissues suggesting that oligomerization could occur at physiological concentration of the receptors. Moreover, both of the receptors showed similar relative affinities for one another indicating that β1AR and β2AR display no preferences for entering either homo- or heterotropic interactions. Thus, when expressed at equimolar concentrations, β1AR and β2AR should form three equal populations of oligomers: β1AR/β2AR hetero-oligomers and two respective types of homo-oligomers [40].

Further studies are aimed to evaluate properties of the receptor complexes in regard to receptor pharmacology and downstream signaling. Binding affinities of nonselective β-adrenergic agonist (isoproterenol) and antagonist (propranolol) remained stable upon the formation of β1AR/β2AR hetero-oligomers. In contrast, changes in binding interactions were detected for subtype specific ligands. For example, the β1AR-selective agonist, xamoterol, showed two separate classes of affinity — minor population of low-affinity and predominant population of high-affinity binding sites. The proportion of the latter was greatly reduced in the presence of β2AR, however the value of inhibition constant remained almost unaltered. Interestingly, co-stimulation of the hetero-oligomer with the β2AR-specific agonist, procaterol, rescued the high-affinity binding site of xamoterol [43].

To assess the functional consequences of the oligomerization-dependent shifts in receptor pharmacology the measurements of adenylyl cyclase activity and extracellular-signal-regulated kinase 1/2 (ERK1/2) phosphorylation were conducted (Fig. 2). Xamoterol successfully stimulated the production of cAMP via β1AR, however this stimulation was lost in the hetero-oligomer. Strikingly, the inability to activate the adenylyl cyclase persisted even in the presence of procaterol, ligand that efficiently induced cAMP accumulation via both β2AR homo-oligomer and β1/β2AR hetero-oligomer. This observation reflects the lack of equivalence between affinity and efficacy. Despite of the restored binding affinity, xamoterol failed to re-establish its functional properties in the presence of the second ligand. Withrespect to nonspecific agonist no significant differences in the ability of isoproterenol to activate the adenylyl cyclase were observed regardless the β1AR and β2AR were expressed individually or together [41,43].

In the case of MAPK signaling the β1AR and β2AR display distinct pattern of activity (Fig. 2). Isoproterenol induced consistent activation of ERK1/2 in cells transiently transfected with β2AR. In contrast, no phosphorylation of ERK1/2 was detected for the agonist-activated β1AR. Interestingly, when both receptors were expressed at the plasma membrane no β-adrenergic-mediated activation of ERK1/2 was observed in response to isoproterenol treatment. These data suggested that the hetero-oligomerization with β1AR inhibited the β2AR-dependent ERK1/2 activation [41].

When considering cellular localization and receptor trafficking both β1AR and β2AR are found primarily at the plasma membrane but only the β2AR undergoes rapid internalization upon agonist stimulation. β1AR resists redistribution from the cell surface to cytoplasm under the same experimental conditions. β1AR/β2AR complex also appears to be resistant to agonist-induced internalization as isoproterenol fails to promote internalization of either the β1AR or the β2AR in cells expressing both receptors [41]. Similar effects have been detected with respect of hetero-oligomerization between β2AR and β3AR. Interaction with β3AR strongly impairs the capacity of β2AR to internalize in an agonist-dependent manner [39]. In this respect, β1AR and β3AR can be described as dominant negative

Table 1. Receptor–receptor interactions of β2AR.

Interaction partner	Trafficking	Pharmacology	Signaling	Reference
α1Dadrenergic receptor (α1DAR)	↑ α1DAR cell surface expression Cross-internalization	↑ α1DAR binding sites density	↑ α1DAR-dependent Ca2 + mobilization	[46]
α2Cadrenergic receptor (α2CAR)	↑ α2CAR cell surface expression	↑ α2cAR binding sites density	↑ α2CAR-dependent ERK phosphorylation	[47]
Adenosine A1receptor (A1AR)	–	No effect on A1AR ligand binding ↑ affinity of β2AR antagonist binding	–	[64]
Angiotensin II type 1 receptor (AT1R)	–	β2AR transinhibition AT1R transinhibition	↓ β2AR ERK activation by AT1R antagonist ↓ AT1R ERK activation by β2AR antagonist	[58]
β1 adrenergic receptor (β1AR)	↓ β2AR internalization	↓ subtype-specific ligand binding No effect on nonspecific ligand binding	↓ β2AR ERK activation No effect on cAMP production	[40–42]
β3 adrenergic receptor (β3AR)	↓ β2AR internalization	No effect on ligand binding	↓ β2AR β-arrestin 2 recruitment ↓ Gi/o coupling No effect on cAMP production	[39]
Bradykinin type 2 receptor (Bk2R)	–	β2AR transactivation	↑ β2AR chloride channel activation by agonist of Bk2R	[62]
Cannabinoid type 1 receptor (CB1R)	↑ CB1R cell surface expression Cross-internalization	↑ Emax and Hill coefficient of WIN (CB1R agonist)-dependent pERK dose–response ↓ Emax and Hill coefficient of WIN (CB1R agonist)-dependent pCREB dose–response	↓ CB1R basal ERK activation ↑ CB1R coupling to Gi/o ↓ CB1R coupling to Gs ↓ β2AR ERK activation by antagonist of CB1	[55]
CXCR4	–	β2AR desensitization	↓ β2AR cAMP production	[57]
δ-Opioid receptor	Cross-internalization	No effect on ligand binding	No effect on cAMP production	[49,50]
κ-Opioid receptor	↓ β2AR internalization	No effect on ligand binding	↓ β2AR ERK activation No effects on cAMP production	[49]
Murine 71 olfactory receptor (M71OR)	↑ M71OR cell surface expression cross-internalization	↑ M71OR binding sites density	↑ M71OR-dependent cAMP production	[48]
Oxytocin receptor (OTR)	–	–	↓ β2AR ERK activation by OTR antagonist ↓ OTR ERK activation by β2AR antagonist ↑ β2AR signaling via PKCζ	[60,61]
Prostaglandin EP1 receptor	No effects on β2AR trafficking	β2AR desensitization	↓ β2AR coupling to Gαs ↓ β2AR-dependent cAMP production	[56]
Somatostatin receptor 5 (SSTR5)	No cross-internalization ↓ β2AR and SSTR5 internalization by combination of β2AR and SSTR5 agonists	–	–	[59]

regulators of agonist-mediated β2AR internalization. Formation of β1AR/β2AR and β3AR/β2AR hetero-oligomers produces novel signaling units that display distinct functional properties compared to individual protomers (Fig. 2).

Crystallographic studies highlight the importance of lipids for receptor–receptor interface in β2AR oligomers [24]. In fact, cholesterol and other components of lipid rafts modulate the physiological function of the β2AR as they are involved in the compartmentalization of the receptor and its signaling machinery [44]. For instance, in cardiomyocytes β2AR localizes specifically to plasma membrane invaginations known as transverse-tubular (T-tubule) regions where the receptor produces spatially restricted signals [45]. In contrast, β1AR is widely distributed at the whole cell surface. Given that β1AR is able to block β2AR-dependent signaling, this type of sequestration may explain why β2AR still triggers the activation of downstream signaling events, even though the expression of β1AR in cardiomyocytes is several folds higher than the expression of β2AR.

3.1 Cell surface expression and α-adrenergic receptors

β2AR can also engage into interactions with some α-adrenergic receptors. It has been demonstrated that β2AR oligomerize with α1DAR [46] and α2CAR [47] but not with α1BAR [14]. The trafficking of α1DAR and α2CAR to the cell membrane is often inefficient and, consequently, facilitates accumulation of non-functional receptors inside the cytoplasm. Screening of more than twenty family A GPCRs revealed that β2AR, but not β1AR nor β3AR, physically associates with α1DAR and α2CAR to rescue their cell surface expression and double their binding sites density [46,47]. This phenomenon of β2AR-driven delivery of receptors to the plasma membrane extends to other GPCRs. For instance, murine 71 olfactory receptor (M71OR) got readily available for binding with its agonists only in cells co-transfected with vector coding for β2AR [48].

3.2 Receptor internalization and opioid receptors

Experiments demonstrating that both δ- and κ-opioid receptors are capable of forming heterodimers with β2AR shed some light on the involvement of oligomerization in the process of receptor internalization [49–51]. It has been previously shown that δ-opioid receptor undergoes rapid endocytosis upon activation mediated by an agonist. In contrast, κ-opioid receptor is not internalized after activation. When co-expressed with δ-opioid receptor, β2AR undergoes not only isoproterenol- but also opioid-mediated internalization. In the same experimental setup, δ-opioid receptor acquires the capacity to internalize in response to β2AR-specific agonist. This data suggests that cross-activation between interacting protomers can occur. On the other hand, co-expression of β2AR with noninternalizing κ-opioid receptor blocks agonist-mediated endocytosis of β2AR and significantly diminishes downstream MAPK activation. This study has indicated that hetero-oligomerization alters GPCR trafficking and may modulate signaling properties of the interacting receptors [49].

Since β2AR and opioid receptors coexist at plasma membrane of cardiac myocyte, treatment with opioids may increase the ratio of β2AR internalization in vivo [49]. This could possibly constitute the rationale for the lack of norepinephrine-mediated activation of β2AR after stimulation with low-dose of opioids in heart muscle cells. However, the physiological relevance of oligomerization between opioid receptors and β2AR was challenged by the study of Ramsay and colleagues who demonstrated that heterotropic interactions with β2AR occurred only at high concentration of the receptor (250,000 copies per cell compared to less than 100,000 copies required for homo-oligomerization between the opioid receptors) [51]. Furthermore, BRET signal for cells co-expressing β2AR and δ-opioid receptor was remarkably weaker when compared with values obtained for δ-opioid homo-oligomerization [50]. This result indicates that formation of opioid receptor homo-oligomers is much more favorable compared to hetero-oligomerization with β2AR when both receptors are expressed at similar levels.

3.3 Signaling pathways and cannabinoid receptors

As the distribution of β2AR overlaps considerably with distributions of other GPCRs, the interactions between these receptors may produce physiologically relevant outcomes. For example, β2AR and CB1 cannabinoid receptor (CB1R) are both expressed in normal tissues of the brain, eye and bone [52], as well as in number of cancer cell lines [53]. In fact, it has been demonstrated that CBRs can modulate β2AR activity [52–54]. Moreover, Hudson and colleagues identified the CB1R as another receptor able to directly associate with β2AR [55]. Besides physical interactions, functional consequences of β2AR/CB1R hetero-oligomerization were found. When expressed alone, CB1R signals via Gαi/o to phosphorylate ERK and acts on Gαs to induce phosphorylation of cyclic AMP response element binding protein (CREB). The presence of β2AR

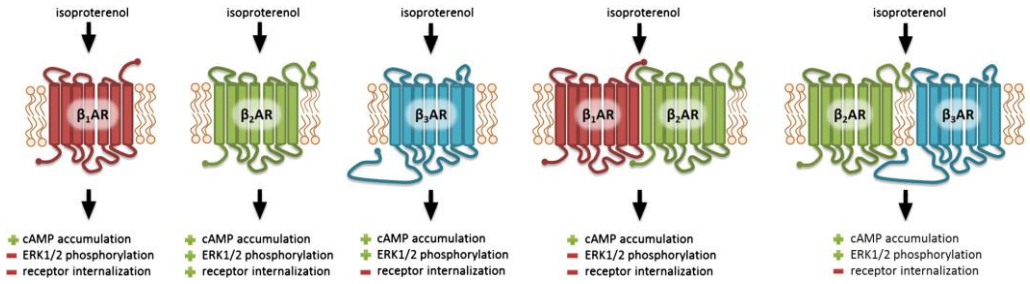

Fig. 2. Effects of isoproterenol on signaling of β-adrenergic receptor hetero-oligomers. Plus signs indicate induction of a signaling. Minus signs state for inhibition or lack of a signaling.

modified this profile of CB1R coupling to G proteins and shifted it towards Gαi/o, thereby resulting in elevated pERK expression and diminished phosphorylation of CREB [55].

3.4 Muscle contractility and myocyte-expressed GPCRs

McGraw and colleagues investigated the interactions between β2AR and prostaglandin EP1 receptor in airway smooth muscles [56]. Although, the EP1 receptor did not significantly affect the contractility of airway smooth muscles, its activation led to significant inhibition of Gαs coupling of its oligomerization partner, β2AR. This, in turn, resulted in a decrease of cAMP production and eventually caused the loss of β2AR-dependent muscle relaxation [56].

Similar pattern of oligomerization-driven inhibition was observed in the case of CXCR4 — the receptor that is expressed in cardiomyocytes where it negatively regulates β2AR-dependent contractility. LaRocca and colleagues demonstrated that this regulation depended on direct receptor–receptor interactions and involved modulation in conformational state of the receptors [57]. In particular, activation of CXCR4 with its endogenous agonist, stromal cell-derived factor-1 (SDF-1α), stabilized the inactive conformation of β2AR, even at the presence of isoproterenol. This was further reflected in SDF-1α-dependent decrease in isoproterenol-evoked cAMP production [57].

Angiotensin II type 1 receptor (AT1R) and β2AR formed a novel signaling unit where blocking of one of the protomers led to the signaling inhibition of the reciprocal one [58]. For instance, increase in phosphorylation of ERK caused by isoproterenol binding to β2AR was abolished by valsartan, blocker of AT1R. Similarly, it was possible to block the angiotensin-induced contractility of cardiomyocytes with antagonists of β2AR [58].

3.5 Other receptors

β2AR/somatostatin receptor 5 (SSTR5) hetero-oligomer displayed interesting pattern of ligand-induced internalization. Stimulation with an agonist of one of the protomers disrupted the oligomer and decreased the cell surface expression of the targeted protomer [59]. For instance, a challenge with β2AR agonist (formoterol) led to β2AR internalization without affecting the SSTR5. However, simultaneous addition of formoterol and SST-14, agonist of SSTR5, blocked the internalization of both β2AR and SSTR5 [59].

Another interesting oligomerization-dependent effect was found when β2AR-OTR interactions were studied [60]. Wrzal and colleagues observed that β2AR signals through PKCζ only when co-expressed with the OTR [61].

Some other GPCRs are capable of oligomerization with β2AR. So far bradykinin type 2 receptor (Bk2R) [62], opsin [63], gastric inhibitory polypeptide receptor [63] and adenosine A1 receptor [64] were demonstrated to interact with β2AR (Table 1). For some of them there is evidence that the interactions may occur in vivo and be physiologically relevant.

4 Concluding remarks

This review illustrates how the methodology and techniques used for studying receptor oligomerization evolved during the past two decades. Initial biochemical studies were rapidly complemented by RET experiments carried out in intact cells to be finally supplemented by tracing of single receptor particles on the surface of plasma membrane. Accumulation of data enabled us to see β2AR oligomers as dynamic and widespread supramolecular complexes. Although, it has been demonstrated that single (i.e. monomeric) β2AR is capable to effectively activate G proteins [65], the abundance of GPCRs accompanying β2AR at plasma membrane of different cell types and large spectrum of potential partners for physical interactions indicate that homo- and hetero-oligomers constitute native state of β2AR. Furthermore, one has to bear in mind that lack of oligomerization between two species of receptors does not block the possibility for the functional cross-talk between them as signaling pathways may cross at multiple levels deep inside the cells.

β2ARs exist predominantly as dimers [66], but at certain conditions they can aggregate and form higher-order oligomers. Some experimental data suggest that these highly packed receptor clusters are impaired in respect of triggering downstream signaling cascades. It is tempting to speculate that formation of higher-order oligomers is in fact a natural overflow-prevention system that attenuates β2AR signaling in the case of excessive receptor production.

Although β2AR is the target for numerous marketed drugs, and it has been well established that this receptor forms both homo- and hetero-oligomers, there is no medication available that has been designed to specifically target the oligomers. Since the design of oligomer-specific compounds is a cumbersome task, the synthesis of bivalent ligands that can interact with two different GPCRs capable of forming oligomers may constitute an interesting alternative [67]. For instance, (R,R′)-4′-methoxy-1-naphtylfenoterol is a potent agonist of β2AR that can also interact with cannabinoid receptors to effectively inhibit proliferation of cancer cells [53,68]. Bivalent ligands of β2AR and A1AR produce biphasic pattern of cAMP production in cells expressing both receptors [69]. We believe that exploiting the phenomenon of receptor oligomerization creates a fascinating approach in drug design and may enable to tweak new compounds to produce complex and highly specific cellular responses.

It is clear that oligomer formation is a crucial aspect of the biological function of β2AR. The consequences of this phenomenon for downstream signaling and for drug design are, however, not fully understood and much work needs to be done to achieve deeper understanding of the β2AR oligomerization.

Acknowledgment

The authors thank Dr. Michel Bernier from the National Institute on Aging for critical reading of the manuscript. The work was supported by a grant (TEAM 2009-4/5) from the Foundation for Polish Science.

References

[1] R. Gasper, S. Meyer, K. Gotthardt, M. Sirajuddin, A. Wittinghofer, Nat. Rev. Mol. Cell Biol., 10 (6) (2009), pp. 423-429

[2] S. Terrillon, M. Bouvier, EMBO Rep., 5 (1) (2004), pp. 30-34

[3] R.A. Hall, Semin. Cell Dev. Biol., 15 (3) (2004), pp. 281-288

[4] S.K. Shenoy, M.T. Drake, C.D. Nelson, D.A. Houtz, K. Xiao, S. Madabushi, E. Reiter, R.T. Premont, O. Lichtarge, R.J. Lefkowitz, J. Biol. Chem., 281 (2) (2006), pp. 1261-1273

[5] L.E. Limbird, R.J. Lefkowitz, J. Biol. Chem., 251 (16) (1976), pp. 5007-5014

[6] W. Krawietz, M. Weinsteiger, M. Pruchniewski, E. Erdmann, Biochem. Pharmacol., 28 (19) (1979), pp. 2999-3007

[7] L.E. Limbird, P.D. Meyts, R.J. Lefkowitz, Biochem. Biophys. Res. Commun., 64 (4) (1975), pp. 1160-1168

[8] N.J. Birdsall, Trends Pharmacol. Sci., 31 (11) (2010), pp. 499-508

[9] C.M. Fraser, J.C. Venter, Biochem. Biophys. Res. Commun., 109 (1) (1982), pp. 21-29

[10] C.M. Fraser, A.R. Kerlavage, A.P. Mariani, J.C. Venter, Proteins, 2 (1) (1987), pp. 34-41

[11] T.E. Hebert, S. Moffett, J.P. Morello, T.P. Loisel, D.G. Bichet, C. Barret, M. Bouvier, J. Biol. Chem., 271 (27) (1996), pp. 16384-16392

[12] A.U. Gehret, A. Bajaj, F. Naider, M.E. Dumont, J. Biol. Chem., 281 (30) (2006), pp. 20698-20714

[13] S. Angers, A. Salahpour, E. Joly, S. Hilairet, D. Chelsky, M. Dennis, M. Bouvier, Proc. Natl. Acad. Sci. U. S. A., 97 (7) (2000), pp. 3684-3689

[14] L. Stanasila, J.B. Perez, H. Vogel, S. Cotecchia J. Biol. Chem., 278 (41) (2003), pp. 40239-40251

[15] Y. Xu, D.W. Piston, C.H. Johnson, Proc. Natl. Acad. Sci. U. S. A., 96 (1) (1999), pp. 151-156

[16] P. Wu, L. Brand, Anal. Biochem., 218 (1) (1994), pp. 1-13

[17] J.J. Fung, X. Deupi, L. Pardo, X.J. Yao, G.A. Velez-Ruiz, B.T. Devree, R.K. Sunahara, B.K. Kobilka, EMBO J., 28 (21) (2009), pp. 3315-3328

[18] J.R. James, M.I. Oliveira, A.M. Carmo, A. Iaboni, S.J. Davis, Nat. Methods, 3 (12) (2006), pp. 1001-1006

[19] M.J. Lohse, Nat. Methods, 3 (12) (2006), pp. 972-973

[20] M. Bouvier, N. Heveker, R. Jockers, S. Marullo, G. Milligan, Nat. Methods, 4 (1) (2007), pp. 3-4

[21] A. Salahpour, B. Masri, Nat. Methods, 4 (8) (2007), pp. 599-600

[22] S. Dorsch, K.N. Klotz, S. Engelhardt, M.J. Lohse, M. Bunemann, Nat. Methods, 6 (3) (2009), pp. 225-230

[23] D. Calebiro, F. Rieken, J. Wagner, T. Sungkaworn, U. Zabel, A. Borzi, E. Cocucci, A. Zurn, M.J. Lohse, Proc. Natl. Acad. Sci. U. S. A., 110 (2) (2013), pp. 743-748

[24] V. Cherezov, D.M. Rosenbaum, M.A. Hanson, S.G. Rasmussen, F.S. Thian, T.S. Kobilka, H.J. Choi, P. Kuhn, W.I. Weis, B.K. Kobilka, R.C. Stevens, Science, 318 (5854) (2007), pp. 1258-1265

[25] B.F. O'Dowd, M. Hnatowich, M.G. Caron, R.J. Lefkowitz, M. Bouvier, J. Biol. Chem., 264 (13) (1989), pp. 7564-7569

[26] T.E. Hebert, T.P. Loisel, L. Adam, N. Ethier, S.S. Onge, M. Bouvier, Biochem. J., 330 (Pt 1) (1998), pp. 287-293

[27] Y. Liang, D. Fotiadis, S. Filipek, D.A. Saperstein, K. Palczewski, A. Engel, J. Biol. Chem., 278 (24) (2003), pp. 21655-21662

[28] W. Guo, E. Urizar, M. Kralikova, J.C. Mobarec, L. Shi, M. Filizola, J.A. Javitch, EMBO J., 27 (17) (2008), pp. 2293-2304

[29] J. Huang, S. Chen, J.J. Zhang, X.Y. Huang, Nat. Struct. Mol. Biol., 20 (4) (2013), pp. 419-425

[30] A. Manglik, A.C. Kruse, T.S. Kobilka, F.S. Thian, J.M. Mathiesen, R.K. Sunahara, L. Pardo, W.I. Weis, B.K. Kobilka, S. Granier, Nature, 485 (7398) (2012), pp. 321-326

[31] A. Salahpour, S. Angers, J.F. Mercier, M. Lagace, S. Marullo, M. Bouvier, J. Biol. Chem., 279 (32) (2004), pp. 33390-33397

[32] J.M. Johnston, H. Wang, D. Provasi, M. Filizola, PLoS Comput. Biol., 8 (8) (2012), p. e1002649

[33] A. Ghosh, U. Sonavane, R. Joshi, Comput. Biol. Chem., 48 (2014), pp. 29-39

[34] M. von Zastrow, B.K. Kobilka, J. Biol. Chem., 267 (5) (1992), pp. 3530-3538

[35] N. Sartania, S. Appelbe, J.D. Pediani, G. Milligan, Cell. Signal., 19 (9) (2007), pp. 1928-1938

[36] T.H. Lan, S. Kuravi, N.A. Lambert, PLoS One, 6 (2) (2011), p. e17361

[37] A. Gavalas, T.H. Lan, Q. Liu, I.R. Correa, J.A. Javitch, N.A. Lambert, Mol. Pharmacol., 4 (3) (2013), pp. 346-352

[38] J. Xu, M. Paquet, A.G. Lau, J.D. Wood, C.A. Ross, R.A. Hall, J. Biol. Chem., 276 (44) (2001), pp. 41310-41317

[39] A. Breit, M. Lagace, M. Bouvier, J. Biol. Chem., 279 (27) (2004), pp. 28756-28765

[40] J.F. Mercier, A. Salahpour, S. Angers, A. Breit, M. Bouvier, J. Biol. Chem., 277 (47) (2002), pp. 44925-44931

[41] C. Lavoie, J.F. Mercier, A. Salahpour, D. Umapathy, A. Breit, L.R. Villeneuve, W.Z. Zhu, R.P. Xiao, E.G. Lakatta, M. Bouvier, T.E. Hebert, J. Biol. Chem., 277 (38) (2002), pp. 35402-35410

[42] W.Z. Zhu, K. Chakir, S. Zhang, D. Yang, C. Lavoie, M. Bouvier, T.E. Hebert, E.G. Lakatta, H. Cheng, R.P. Xiao, Circ. Res., 97 (3) (2005), pp. 244-251

[43] C. Lavoie, T.E. Hebert, Can. J. Physiol. Pharmacol., 81 (2) (2003), pp. 186-195

[44] Y. Xiang, V.O. Rybin, S.F. Steinberg, B. Kobilka, J. Biol. Chem., 277 (37) (2002), pp. 34280-34286

[45] V.O. Nikolaev, A. Moshkov, A.R. Lyon, M. Miragoli, P. Novak, H. Paur, M.J. Lohse, Y.E. Korchev, S.E. Harding, J. Gorelik, Science, 327 (5973) (2010), pp. 1653-1657

[46] M.A. Uberti, C. Hague, H. Oller, K.P. Minneman, R.A. Hall, J. Pharmacol. Exp. Ther., 313 (1) (2005), pp. 16-23

[47] S.C. Prinster, T.G. Holmqvist, R.A. Hall, J. Pharmacol. Exp. Ther., 318 (3) (2006), pp. 974-981

[48] C. Hague, M.A. Uberti, Z. Chen, C.F. Bush, S.V. Jones, K.J. Ressler, R.A. Hall, K.P. Minneman, Proc. Natl. Acad. Sci. U. S. A., 101 (37) (2004), pp. 13672-13676

[49] B.A. Jordan, N. Trapaidze, I. Gomes, R. Nivarthi, L.A. Devi, Proc. Natl. Acad. Sci. U. S. A., 98 (1) (2001), pp. 343-348

[50] M. McVey, D. Ramsay, E. Kellett, S. Rees, S. Wilson, A.J. Pope, G. Milligan, J. Biol. Chem., 276 (17) (2001), pp. 14092-14099

[51] D. Ramsay, E. Kellett, M. McVey, S. Rees, G. Milligan, Biochem. J., 365 (Pt 2) (2002), pp. 429-440

[52] B.D. Hudson, T.E. Hebert, M.E. Kelly, Mol. Pharmacol., 77 (1) (2010), pp. 1-9

[53] R.K. Paul, A. Ramamoorthy, J. Scheers, R.P. Wersto, L. Toll, L. Jimenez, M. Bernier, I.W. Wainer, J. Pharmacol. Exp. Ther., 343 (1) (2012), pp. 157-166

[54] J. Tam, V. Trembovler, V. Di Marzo, S. Petrosino, G. Leo, A. Alexandrovich, E. Regev, N. Casap, A. Shteyer, C. Ledent, M. Karsak, A. Zimmer, R. Mechoulam, R. Yirmiya, E. Shohami, I. Bab, FASEB J., 22 (1) (2008), pp. 285-294

[55] B.D. Hudson, T.E. Hebert, M.E. Kelly, Br. J. Pharmacol., 160 (3) (2010), pp. 627-642

[56] D.W. McGraw, K.A. Mihlbachler, M.R. Schwarb, F.F. Rahman, K.M. Small, K.F. Almoosa, S.B. Liggett, J. Clin. Invest., 116 (5) (2006), pp. 1400-1409

[57] T.J. LaRocca, M. Schwarzkopf, P. Altman, S. Zhang, A. Gupta, I. Gomes, Z. Alvin, H.C. Champion, G. Haddad, R.J. Hajjar, L.A. Devi, A.D. Schecter, S.T. Tarzami, J. Cardiovasc. Pharmacol., 56 (5) (2010), pp. 548-559

[58] L. Barki-Harrington, L.M. Luttrell, H.A. Rockman, Circulation, 108 (13) (2003), pp. 1611-1618

[59] R.K. Somvanshi, N. Chaudhari, X. Qiu, U. Kumar, J. Mol. Signal., 6 (2011), p. 9

[60] P.K. Wrzal, D. Devost, D. Petrin, E. Goupil, C. Iorio-Morin, S.A. Laporte, H.H. Zingg, T.E. Hebert, Cell. Signal., 24 (1) (2012), pp. 342-350

[61] P.K. Wrzal, E. Goupil, S.A. Laporte, T.E. Hebert, H.H. Zingg, Cell. Signal., 24 (1) (2012), pp. 333-341

[62] K.K. Haack, M.R. Tougas, K.T. Jones, S.S. El-Dahr, H. Radhakrishna, N.A. McCarty, J. Biomol. Screen., 15 (3) (2010), pp. 251-260

[63] M. Vrecl, L. Drinovec, C. Elling, A. Heding, J. Recept. Signal Transduct. Res., 26 (5–6) (2006), pp. 505-526

[64] P.C. Chandrasekera, T.C. Wan, E.T. Gizewski, J.A. Auchampach, R.D. Lasley, Cell. Signal., 25 (4) (2013), pp. 736-742

[65] M.R. Whorton, M.P. Bokoch, S.G. Rasmussen, B. Huang, R.N. Zare, B. Kobilka, R.K. Sunahara, Proc. Natl. Acad. Sci. U. S. A., 104 (18) (2007), pp. 7682-7687

[66] K. Herrick-Davis, E. Grinde, A. Cowan, J.E. Mazurkiewicz, Mol. Pharmacol., 84 (4) (2013), pp. 630-642

[67] C. Valant, J. Robert Lane, P.M. Sexton, A. Christopoulos, Annu. Rev. Pharmacol. Toxicol., 52 (2012), pp. 153-178

[68] R.K. Paul, A. Wnorowski, I. Gonzalez-Mariscal, S.K. Nayak, K. Pajak, R. Moaddel, F.E. Indig, M. Bernier, I.W. Wainer, Biochem. Pharmacol., 87 (4) (2014), pp. 547-561

[69] P. Karellas, M. McNaughton, S.P. Baker, P.J. Scammells, J. Med. Chem., 51 (19) (2008), pp. 6128-6137