Disrupting GPCR Complexes with Smart Drug-like Peptides

Subjects: Pharmacology & Pharmacy

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G protein-coupled receptors (GPCRs) are a superfamily of proteins classically described as monomeric transmembrane (TM) receptors. However, increasing evidence indicates that many GPCRs form higher-order assemblies made up of monomers pertaining to identical (homo) or to various (hetero) receptors. The formation and structure of these oligomers, their physiological role and possible therapeutic applications raise a variety of issues that are currently being actively explored. In this context, synthetic peptides derived from TM domains stand out as powerful tools that can be predictably targeted to disrupt GPCR oligomers, especially at the interface level, eventually impairing their action.

peptide therapeutics transmembrane peptides GPCR oligomers non-natural amino acids cyclic peptides retro-enantio

1. Introduction

G protein-coupled receptors (GPCRs) constitute the largest and most versatile superfamily of cell membrane-bound proteins, made up of seven trans-membrane α-helices (TM1 to TM7) [1][2][3] connected by intracellular (IL-1 to IL-3) and extracellular loops (EL-1 to EL-3), and coupled to an intracellular heterotrimeric G protein (e.g., Gs, Gi/o, Gq/11, G12/13) [4]. GPCRs are commonly grouped into six subfamilies (A-F) [5], based on sequence homology and functionality. Despite this apparent diversity, all GPCRs mediate their effects upon agonist-induced activation of the receptor at the extracellular site by a wide variety of ligands and then transduce the signal into intracellular responses [6]. Endogenous GPCR agonists are physically and chemically very diverse, including neurotransmitters (i.e., dopamine, serotonin), hormones (i.e., estrogen, angiotensin), proteins (i.e., chemokines), odors, photons, lipids (i.e., anandamide) or peptides (i.e., bradykinin), among many others [7]. Moreover, and more interestingly, ligand affinity for the GPCR primary (orthosteric) site and efficacy of activation can be increased or decreased by other effectors that bind to a separate (allosteric) site [8].

Given that GPCR signaling is involved in a diverse number of biological processes, GPCRs are considered ideal therapeutic targets [9] for a wide assortment of human diseases ranging from allergic rhinitis to pain, type-2 diabetes mellitus, obesity, depression, insomnia or cancer, to name just a few [10][11][12]; indeed, 34% of currently FDA-approved small-molecule drugs bind to GPCRs [13]. Originally described as cell-surface monomers that form a ternary complex with the extracellular ligand and the intracellular G protein [14], GPCR higher-order oligomers have in recent years been increasingly recognized as novel signaling units with functional properties distinct from their

constituent receptors, thus opening up a new, only sparingly explored area of study within the GPCR field [15][16]. One possible strategy to probe into GPCR oligomerization and its impact on health conditions would consist in interfering in complex formation by means of exogenous synthetic peptides replicating TM domains involved in helix–helix interactions [17].

2. GPCR Oligomers

The human genome encodes nearly 1000 different GPCRs, each one highly specific to a signaling pathway [18]. However, growing evidence indicates that many GPCRs can form active higher-order oligomers constituted by equal (homo) or different (hetero) monomers [19][20][21][22][23][24][25][26], with functional properties distinct from their protomer components [27] and generally involved in both healthy and pathological processes [28], thus making them ideal targets for the development and screening of novel drugs [29][30].

One of the first reported GPCR oligomers involved δ- and κ-opioid receptors that, when co-expressed, formed a stable heterodimer with properties not found in cells expressing the same receptor monomers [31]. Subsequently, many other GPCR homo- and/or hetero-complexes have been unveiled, often displaying unique characteristics.

In many of these investigations the importance of TM helices in GPCR oligomerization has been demonstrated, portraying the GPCR complexes as dynamic species in which activation by the agonist induces a realignment of TM dimerization interfaces [32][33]. Indeed, it has been found that a dynamic equilibrium between monomeric and dimeric species can take place [34], modulated by ligand binding, which in turn can enhance or decrease heteromer interaction [35]. Therefore, while the minimal GPCR functional unit can be regarded as constituted by one monomeric receptor and one heterotrimeric G protein (1:1) [36], GPCR dimers can occur when: (i) two G proteins bind both dimer protomers (2:2) [37][38] or (ii) one G protein binds one protomer in the dimer (1:2) [39].

Another distinctive feature of some GPCRs is the switching of the G protein-coupled protomer when dimerization occurs. For instance, serotonin $5HT_{2A}R$ couples Gq; however, heteromer formation by cannabinoid CB_1R and $5HT_{2A}R$ makes both receptors signal via Gi [40] (**Figure 1**A). In other words, some GPCR heteromers can couple G protein species different from those favoured by their protomers. Other reported examples are: (i) a heterodimer formed by dopamine D_1 and D_2 receptors that couples Gq instead of Gs or Gi [41] (**Figure 1**B); (ii) the heteromer formed by angiotensin AT_1 and α_{2c}-adrenergic receptors couples Gs instead of Gi or Gq [42]; and (iii) a melatonin MT_1-MT_2 receptor dimer that couples Gq instead of Gi [43].

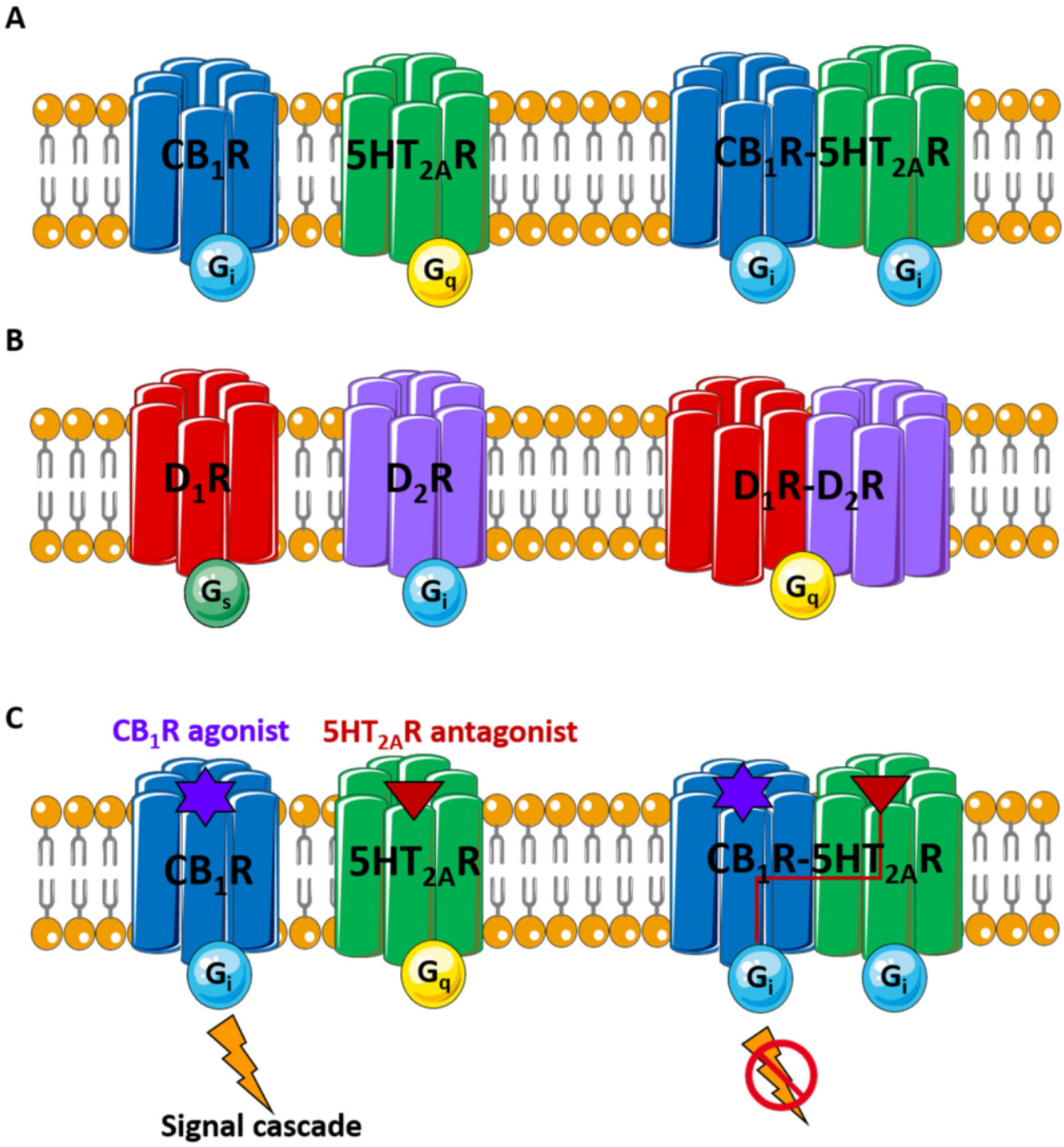

Figure 1. (**A**) The serotonin $5HT_{2A}R$ and the cannabinoid CB_1R monomers couple Gi and Gq proteins, respectively; when dimerized, however, $5HT_{2A}R$ switches Gq protein with Gi; (**B**) The dopamine D_1R and D_2R monomers couple Gs or Gi, respectively; however, the heterodimer D_1R-D_2R couples Gq; (**C**) The serotonin $5HT_{2A}R$ antagonist blocks the signal activation of the cannabinoid CB_1R agonist when dimerized.

Functionally, GPCR complexes can cause a positive or negative cooperation between promoters, i.e., ligand one binds to protomer one, enhancing or inhibiting, respectively, the affinity of ligand two for protomer two [44]. In general, intermolecular communication between GPCR homo- and heteromers tends to produce synergistic responses (i.e., functional cross-talk) [45]. A more singular phenomenon is cross-antagonism (**Figure 1**C), which occurs when a protomer antagonist blocks the signal activation of the other protomer [25][40][45]. Such a situation

has been described for some GPCR complexes, including the metabotropic Gb_1-Gb_2 receptors [46], opioid δ-μ receptors [47], somatostatin SST_5-dopamine D_2 receptors [48], adenosine A_{2A}-dopamine D_1 receptors [49], orexin-corticotropin-releasing factor receptor [50] or angiotensin II AT_1/dopamine D_2 receptor [51].

Despite the extensive literature on GPCR oligomers, in most cases the assessment of their functionality has been only partially addressed and needs further investigation. In this context, chimeric peptide constructs have shown the ability to disrupt homo- and heteromer complexes, altering agonist-induced functionality and providing knowledge on the physiological role of GPCR receptor–receptor interactions [52][53][54][55].

3. Synthetic TM Peptides as Tools for GPCR Complex Exploration

The identification of protein–protein interaction interfaces constitutes a fundamental aspect in the study of GPCR complex formation [56], in that it can expand the understanding of the role that receptor oligomerization plays in intercellular communication or in some pathological conditions.

Increasing evidence indicates that specific TM helices are required for oligomerization, and that the synthetic peptides reproducing them are powerful tools to identify sequences essential for GPCR complexation and, by blocking their assembly, gain insights into the functional role of the complex [52][57][58].

For instance, Köfalvi et al. (2020) have recently studied how the adenosine-cannabinoid receptors, specifically the A_{2A}R-CB_1R heterotetramer interface, which also includes A_{2A}R-A_{2A}R and CB_1R-CB_1R homodimers, is established. To this end they have used computational modelling, with input from several biophysical and biochemical techniques, to design TM interference peptides reproducing each of the A_{2A}R and CB_1R TM1-7 helices. The synthetic versions, fused to the cell-penetrating HIV-Tat sequence, were tested by in vitro bimolecular fluorescence complementation (BiFC) experiments. Peptides replicating TM5 and TM6 of both receptors were able to disrupt the heterotetramer; thus, the involvement of their interfaces in the complex formation was confirmed. On the other hand, in the absence of the CB_1R receptor, BiFC assays showed that the A_{2A}R-A_{2A}R homodimer was only disrupted by peptide A_{2A}R TM6, while when A_{2A}R was missing, CB_1R TM4 was the only peptide disturbing CB_1R-CB_1R homodimer formation, altogether indicating that TM6 and TM4 sequences are involved in A_{2A}R and CB_1R homodimer interfaces, respectively [59].

Once the interfering peptides are identified, they can be used to investigate GPCR complex implications in numerous physiopathological disorders. As an example, Borroto-Escuela et al. (2018) found that rat A_{2A}R TM5 peptide microinjection into the nucleus accumbens causes A_{2A}R-D_2R heteromer dissolution plus abrogation of the inhibitory effects of the A_{2A}R agonist CGS21680 on cocaine self-administration, therefore confirming that the A_{2A}R-D_2R hetero-complex can be used as a novel target to treat cocaine disorders [53].

More examples where synthetic peptides replicating TM helices involved in dimerization have been shown to be able to split GPCR complex formations are included in **Table 1**. The in vitro (biophysical and/or biochemical) and in

vivo assays used to confirm the existence of GPCR dimers in live cells and their implication (if known) in health disorders, are also presented.

Table 1. GPCR complexes disrupted by synthetic TM peptides.

GPCR Complex	TMs Involved in Dimerization	Synthetic TM Disruptor Peptide	In Vitro/In Vivo Assays Performed	Patho-Physiological Implication	Ref.
$A_{2A}R$-D_2R	TM4/5 interface	$A_{2A}R$ TM5	• BRET • PLA • Cocaine self-administration	Cocaine use	[53]
APJR-OX_1R	TM4/5 interface	APJ TM4, TM5	• BRET • Co-IP	-	[60]
APJR homodimer	TM1, TM2, TM3, TM4	TM1, TM2, TM3, TM4	• BRET • FRET • TIRFM • Co-IP	-	[61]
$A_{2A}R$-CB_1R	TM 5/6 interface	CB_1R TM5 TM6 $A_{2A}R$ TM5 TM6	• BiFC • BRET • CODA-RET • Glutamate release	Glutamate release	[59]
A_1R-$A_{2A}R$	TM 5/6 interface	$A_{2A}R$ TM4, TM5, TM6	• BiFC	Neurodegeneration	[62]

GPCR Complex	TMs Involved in Dimerization	Synthetic TM Disruptor Peptide	In Vitro/In Vivo Assays Performed	Patho-Physiological Implication	Ref.
		A_1R TM5 and TM6	• PLA • BRET • cAMP production • DMR	Neuroinflammation	
CB_1R-$5HT_{2A}R$	TM 5/6 interface	CB_1R TM5, TM6	• BRET • PLA • BiFC • NORT • Hot plate test	Cognitive impairment	[40]
M_3R homodimer	TM1, TM5, TM7	TM1-TM5-TM7	• BRET	-	[63]
CCKR homodimer	TM6	TM6	• BRET • FRET	-	[64]
CCR5 homodimer	TM1, TM2, TM4	TM1, TM4	• FRET Calcium determination	-	[65]
RhoR homodimer	TM1,TM2, TM4, TM5, H8	TM1, TM2, TM4, TM5	• BRET • cAMP production	Phototransduction	[66]

GPCR Complex	TMs Involved in Dimerization	Synthetic TM Disruptor Peptide	In Vitro/In Vivo Assays Performed	Patho-Physiological Implication	Ref.
β_2AR homodimer	TM1, TM5, TM6, H8	TM6	• Adenylyl cyclase activity • Densitometric analyses	-	[17]
SCTR	TM4	TM4	• FRET • BRET	Liver diseases	[55]
AT1aR-SCTR	TM1/2 interface TM4/4 interface	AT1aR TM1, TM4 SCTR TM2, TM4	• BRET • FRET • cAMP	Hyperosmolality-induced drinking	[54]
FZD_6 homodimer	TM4, TM5	TM4, TM5	• FRAP • FCCS	Cancer and neurologic disorders	[67]
MOR-DOR	MOR TM1	MOR TM1	• Co-IP • Immunoblotting • Tail immersion	Morphine tolerance	[68]

Abbreviations: $5HT_{2A}R$, serotonin receptor type 2 A; A_1R, adenosine receptor type 1; $A_{2A}R$, adenosine receptor type 2A; APJR, apelin receptor; AT1aR, angiotensin receptor type 1a; BiFC, bimolecular fluorescence complementation; BRET, bioluminescence resonance energy transfer; cAMP, cyclic adenosine monophosphate; CB1R, cannabinoid receptor type 1; CCKR, cholecystokinin receptor; CCR5, chemokine receptor type 5; CODA-RET, complemented donor-acceptor resonance energy transfer; Co-IP, co-immunoprecipitation; D_2R, dopamine receptor type 2; DMR, dynamic mass redistribution; DOR, δ-opioid receptor; FCCS, fluorescence cross-correlation

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spectroscopy; FRAP, fluorescence recovery after photobleaching; FRET, fluorescence resonance energy transfer; FZD_8, frizzled-8 receptor; M_3R, muscarinic acetylcholine receptor type 3; MOR, μ-opioid receptors; NORT, novel object recognition test; OX_1R, orexin receptor type 1; PLA, proximity ligation assay; RhoR, rhodopsin receptor; SCTR, secretin receptor; TIRF, total internal reflection fluorescence; β_2AR, adrenergic receptor type β_2.

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