G protein-coupled receptor-biased signaling: potential drug discovery to facilitate treatment of metabolic diseases

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ABSTRACT

G protein-coupled receptors (GPCRs) are important, potential drug targets for the treatment of metabolic disorders, such as obesity. GPCRs crosstalk with several transducers, including heterotrimeric G proteins, GPCR kinases (GRKs), and β-arrestins. GPCR-biased agonism has raised the potential of novel drug development to preferentially activate therapeutic signaling pathways over pathways that lead to unwanted side effects. The obesity epidemic and its metabolic complications continue to be a major global public health threat but effective treatments are limited. The accelerated development of structural techniques, like X-ray crystallography and cryo-electron microscopy, has paved the way to understanding how biased agonism measured at GPCRs results in specific downstream physiologic responses. Herein some well-validated GPCR targets are briefly summarized and several new and promising receptors for obesity treatment are outlined. This review highlights the significance of deciphering the role of GPCRs in obesity pathology and biased signaling for drug development. We anticipate the review will facilitate the development of novel GPCR-targeted anti-obesity drugs that lead to heightened therapeutic efficacy with decreased side effect profiles.

Keywords: GPCR, biased signaling, obesity, energy homeostasis, drug target

1. INTRODUCTION

An obese or overweight body habitus is defined as excess body fat mass due to a chronic accumulation of energy intake over energy expenditure. A body mass index (BMI) > 25 kg/m\textsuperscript{2} and > 30 kg/m\textsuperscript{2} is considered overweight and obese, respectively. Obesity increases the risk of diseases, such as cancer, cardiovascular diseases, and type 2 diabetes, and complicates the management of many diseases [1–4]. Generally, factors accounting for increasing obesity prevalence involve the intake of energy-dense food with a reduction in physical activity [5], as well as chronic stress, circadian desynchronization [6], and sleep deprivation [7].

The global obesity prevalence is increasing and has nearly tripled since 1975. This phenomenon has caused healthcare providers to focus on obesity management, including lifestyle improvement and medication development [4]. Notably, short-term behavioral interventions are not ideal for long-term weight loss. In addition, lifestyle interventions only provide moderate efficacy against obesity. Drug discovery for the pharmacologic management of obesity has been extremely challenging and failed due to safety concerns [8]. Most of the previously approved anti-obesity drugs have been withdrawn because of adverse cardiovascular effects (sibutramine, dexfenfluramine, and rainbow pills), increased risk of depression (rimonabant), or drug addiction and abuse (methamphetamine) [8]. Among the small molecule drugs, only phentermine is not associated with adverse cardiovascular effects and can be used long-term as an anti-obesity drug [8].
Since the discovery of leptin, a mechanistic understanding of energy homeostasis has been achieved, but how to translate the leptin studies to clinical use has not been determined. The central nervous system controls appetite and systemic energy metabolism but direct modulation of these signaling pathways needs specific and selective targeting of cellular circuits, which is difficult. Recently, clinical trials involving therapeutic agents targeting the glucagon-like peptide 1 receptor (GLP1R), such as semaglutide, indicated drug-based management of metabolic diseases, including obesity, may be forthcoming [9–11]. To balance food intake and energy expenditure, novel drugs targeting the neuroendocrine system mediate bidirectional crosstalk between the central nervous system and the periphery. Understanding the molecular mechanism that balances appetite and energy expenditure provides insight into metabolic disease drug discovery [12].

G protein-coupled receptors (GPCRs) are important membrane proteins for transducing signals involving ions, odorants, hormones, neurotransmitters, and other stimuli from the extracellular matrix into the cell [13]. The classic signal transduction through GPCRs is dependent on activation of heterotrimeric G proteins, which are composed of three subunits (Gα, Gβ, and Gγ). Interestingly, only four G-protein families have been classified (Gs, Gi/o, Gq/11, and G12/13). The G-protein family couples to diverse receptors, which is then translated into diverse physiologic effects. A conserved mechanism exists for receptor-catalyzed G-protein activation that involves alterations in the nucleotide-bound state of the Gα subunit [14]. Arrestins are responsible for desensitizing GPCRs. Compared to G protein diversity, there are only four arrestins widely distributed in a variety of tissues. Generally, phosphorylation of GPCRs by GPCR kinases (GRKs) desensitizes G-protein signaling and promotes arrestin recruitment [15]. Biased agonism is primarily focused on defining if the ligand is biased towards G protein or arrestin. A full agonist that activates multiple pathways and leads to side effects is referred to as a balanced ligand. Likewise, a drug that increases or biases activity transduction of a specific signaling pathway is referred to as a biased ligand [16].

Biased agonism is a broad concept that attempts to implement agonist use in clinical practice. Structural studies of various GPCRs and molecular dynamics of GPCRs in aqueous solutions indicate how GPCRs mediate biased signaling. Biased agonism fits into functional selectivity, which may be afforded using a single agonist. A classic example is the dopamine receptor discovered by Rashid and co-workers. Specifically, the dopamine receptors, D1 and D2, have been shown to be coupled to Gs and Gi proteins, respectively, according to the International Union of Basic and Clinical Pharmacology (IUPHAR). D1 and D2 can form a heterodimer which do not couple to Gs or Gi proteins. Instead, the dimer couples to Gq, which activates cAMP and calcium signals [17]. In addition to G protein selectivity, signaling bias of GPCRs involves bias on G protein or arrestin triggering.

The basis for signaling bias involves the ability of ligands to stabilize active conformations of a GPCR, which are distinct from stabilization by other agonists that transduce different signaling pathways, such as GRKs or β-arrestins [18]. Drug development based on signaling bias leads to the discovery of novel and more effective drugs with fewer adverse effects [19]. A classic example of signaling bias is G protein-biased agonism on μ-opioid receptors, which would yield safer analgesic therapeutics because induction of respiratory depression through β-arrestin signaling would be less likely [20]. Thus, GPCR-biased signaling provides hope for drug discovery and development that improves energy expenditure or metabolic homeostasis and has higher potency while avoiding adverse effects.

Given the possible therapeutic effect of modulating GPCR signaling pathways in obesity, we summarized the roles of food intake-related gut hormones and their receptors involving ghrelin, glucagon-like peptide 1 (GLP1), cholecystokinin (CKK), peptide tyrosine tyrosine (PYY), apelin, α-melanocyte-stimulating hormone (α-MSH), and cannabinoid-1 receptor. The potential drug leads against these receptors are summarized in Table 1. We speculate that these findings will provide insight into novel drug discovery against GPCRs with potential therapeutic benefits in obesity treatment.

2. ROLES OF ENDOGENOUS GHERELIN ON ENERGY HOMEOSTASIS

Ghrelin, also called the hunger hormone, is a gastric peptide hormone related to food intake, body weight, and taste sensation. Ghrelin is an orexigenic peptide hormone secreted from the stomach in response to hunger, which in turn stimulates the ghrelin receptor in the brain to initiate appetite [33, 34]. The ghrelin receptor, growth hormone secretagogue receptor (GHSR), is a class A GPCR that couples to Goq/11, Ga1o, and Gq11/13, as well as β-arrestin-based scaffolds [35]. Thus, ghrelin receptor activation transduces biased signals for diverse physiologic responses. Ghrelin receptor coupling to Gi and Gq has been elucidated by cryo-EM technology [36, 37]. Compared to the ghrelin receptor conformations coupled to Gq and Gi, only minor conformational differences were demonstrated (Figure 1).

In addition to the orthosteric binding pocket composed of the ghrelin receptor transmembrane helices (TM) 3, 4, 5, 6, and 7, acyl-ghrelin occupies an extended pocket involved in the second extracellular loop (ECL2). A mouse line in which the ghsr gene was deleted in all tissues was established for understanding the roles of GHSR, which exhibited improved insulin sensitivity, elevated energy expenditure, and reduced body fat mass [38]. Furthermore, neuronal ghsr knock-out mice were shown to have increased energy expenditure and thermogenesis without decreasing food intake, which
indicated that suppressing central ghrelin signaling may shed light on a novel anti-obesity strategy that simultaneously boosts fat combustion and physical activity [39]. Notably, the mouse Gq/11 protein was shown to inversely correlate with UCP1 expression in brown adipose tissue (BAT), further reducing whole-body energy expenditure [40]. The result indicated that inhibition of Gq signaling may be a novel therapeutic strategy for obesity.

As mentioned above, GHSR activation transduces Gq or Gi signaling, which depends on ligand selectivity. Because the ghrelin receptor displays a high basal activity representing 50% of its maximal activity [41], reducing the basal activity of the ghrelin receptor by an inverse agonist may be essential in developing anti-obesity agents. Previous studies have mined GHSR ligands and showed inverse agonism, such as [D-Arg1,D-Phe5,D-Trp7,9,Leu11]-substance P [42], PF-5190457 [43], and N-terminal fragment of liver-expressed antimicrobial peptide 2 (LEAP2) [44]. Among these ligands, PF-5190457 has progressed to a phase I clinical trial for type 2 diabetes treatment (NCT01522807 and NCT01247896). The crystal structure of GHSR bound to PF-5190457 and the cryo-EM structure of the ghrelin-GHSR-Go complex was determined, which revealed the GHSR inverse agonism binding mode [45]. As shown in the GHSR complex bound to PF-5190457 (PDB ID: 7F83), TM6 displays an outward shift, which enlarges the orthosteric pocket of GHSR and shows that the inactive conformation differs from the conformation of ghrelin-GHSR (Figure 1). In the GHSR conformation activated by ghrelin, a polar interaction network formed by E1243.33, R2836.55, S2175.43, and N305 7.35 is necessary for receptor activation, as shown by a mutagenesis study and functional assay (Figure 1). Notably, S2175.43 and R2836.55 were observed in the agonist-bound conformation but not in the antagonist-bound structure. A bifurcated ligand-binding pocket was separated by a salt bridge between E1243.33 and R2836.55 in the interaction of PF-5190457 and GHSR. In addition, F119 3.28 and Q1203.29 are essential for inverse agonism. Hydrogen bonds formed by PF-5190457 and D992.60 and S3087.38 of the GHSR are key facets for inverse agonist recognition by the GHSR. The bottom of the binding pocket is composed of F2796.51, W2766.48, and F3127.42. The hydrophobic clusters are also important for antagonism of GHSR by compound 21 [46]. LEAP2 is an endogenous antagonist of GHSR that is secreted from the liver and intestines that has been shown to fully inhibit GHSR activation by ghrelin and block the physiologic effects, such as food intake, growth hormone release, and blood glucose elevation [47]. LEAP2 and its N-terminal portion exhibit GHSR1a inverse agonism, which competitively antagonizes ghrelin-induced calcium mobilization and inositol-1-phosphate (IP1) production [44], revealing activity on Gi and Gq signaling. A randomized

<table>
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<th>Ligand</th>
<th>Ligand type</th>
<th>Receptor</th>
<th>Signaling bias</th>
<th>Development status</th>
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Table 1 | Therapeutic agents with biased signaling approved or in clinical trials.
controlled trial moved one step forward to the clinical use of LEAP2 by showing that LEAP2 intervention suppresses ad libitum food intake and meal duration but not a liquid-mixed meal without additional hunger [48]. Even though further pharmaceutical and clinical exploration is needed, another step towards the clinical pharmacotherapy targeting the ghrelin-GHSR pathway for obesity, type 2 diabetes, and other related metabolic diseases was achieved [49].

Based on the GHSR structural information and biological studies, novel development of non-peptide small molecules targeting GHSR-biased signaling has been carried out [25], such as JMV1843, AZ-GHS-38, and JMV3002.

As mentioned above, ghrelin is a hunger hormone but ghrelin mediates diverse physiologic actions, including growth hormone release, gastric motility, reward behavior, and emotion. Developing these non-peptide agents...
is necessary for avoiding the on-target side effects. The discovery of small molecules with biased signaling can distinguish between ghrelin responses.

### 3. GLUCAGON-LIKE PEPTIDE 1 (GLP-1) RECEPTOR AGONISTS FOR GLUCOSE AND ENERGY METABOLISM

GLP-1 receptor is an established drug target against metabolic diseases. GLP-1 receptor belongs to class B GPCRs and is coupled to Gs protein. GLP-1 receptor is characterized by having long, extracellular N-terminal domains. GLP-1 receptor is important in the secretion of insulin. Thus, GLP-1 agonists are widely considered for drug development in the treatment of diabetes and obesity. Despite clinical success, GLP-1 receptor peptide agonists are suboptimal due to administration and side effect profiles, including nausea and vomiting. For peptide GLP-1, mutagenesis has been carried out to clarify the signaling profiles, aiming to avoid the adverse effects in future studies. Mutating any single residue of GLP-1 (7-36) to alanine results in a loss of ability to lower blood glucose. With T11A or S14A, the mutant reduces β-arrestin recruitment approximately 10-fold. With S17A or S18A, ERK1/2 phosphorylation increases β-arrestin recruitment approximately 5-fold. Mutations of three serines silence ERK1/2 phosphorylation while mutations of all four residues abolish β-arrestin 2 recruitment, ERK1/2 phosphorylation, and calcium mobilization but still stimulate cAMP and insulin secretion in mice and cells. These findings suggest that hydrogen bonding controls cell signaling, as well as an important regulatory hydroxyl patch in class B GPCR hormones.

Several non-peptide GLP-1 receptor agonists have been identified, including PF-0688296, glatuzumab, semaglutide, and TT-OAD2. TT-OAD2 binding has very limited overlap with full length GLP-1 and the biased agonist, exendin-P5 (ExP5). Therefore, this compound series may modulate peptide activity in the physiologic response. It has been shown that TT-OAD2 inhibits cAMP, calcium mobilization, ERK1/2 phosphorylation, and β-arrestin responses mediated by GLP-1 and oxyntomodulin. This phenomenon indicates that the presence of TT-OAD2 probably inhibits all endogenous peptide effects at higher concentrations, biasing receptor responses primarily to cAMP production mediated by the compound, and the signaling transduced by endogenous peptide may still occur when the compound is at a lower concentration. Similarly, TTP273 has been reported to display greater efficacy at lower levels, revealing the basal physiologic responses induced by the endogenous peptide ligands may be important for clinical effects. ERK1/2 phosphorylation is not the only measurement for β-arrestin recruitment, although β-arrestin is required for ERK1/2 and CREB activation at pharmacologic doses of GLP-1. During GLP-1R activation, β-arrestin-2 (ARRB2) has a role in uncoupling G-proteins and recruits additional pathways, including phosphatidylinositol 3-kinase (PI3K), focal adhesion kinase (FAK), and ERK1/2 phosphorylation. ARRB2 knockout mice were shown to have a minor role in GLP-1R internalization. In addition,
ARRB2 has been shown to contribute to partial uncoupling of cAMP/PKA signaling in the physiologic concentration range of GLP1, which leads to a reduction in insulin secretion [60].

Biased agonism at the GLP-1R stimulates cAMP over β-arrestin recruitment, resulting in less receptor internalization. However, GLP-1R agonism or glucose-dependent insulinotropic polypeptide receptor (GIPR) infusion causes a heart rate increase [62, 63]. Combined GLP-1R/GIPR agonism appears to induce fewer side effects. Tirzepatide is a synthetic, linear peptide composed of 39 amino acids and has a half-life of approximately 5 days, which is suitable for weekly dosing [64]. A phase II clinical trial involving tirzepatide in type 2 diabetes patients reported a significant reduction in body weight (5%-10%) alongside substantial reductions in waist circumference following a 12-week administration [65]. GCGR agonism has a positive chronotropic and inotropic action on the heart [66]. To maximize the drug efficacy and mitigate safety, tri-agonists (GLP-1R/GCGR/GIPR) have been developed. A phase II trial involving the tri-agonist, retatrutide, showed a dose-dependent increase in heart rate that peaked at 24 weeks and declined thereafter; treatment for 48 weeks resulted in a substantial reduction in body weight [67].

4. CHOLECYSTOKININ A RECEPTOR (CCKAR): AN ATTRACTIVE DRUG TARGET FOR METABOLIC DISEASES

The CCKAR belongs to class A GPCRs that are recognized by CCK, which regulates nutrient homeostasis. The CCKAR is distributed in the pylorus, pancreas, and gallbladder, contributing to micelle formation, stimulating lipolytic and proteolytic enzymes delivery, and modulating the delivery rate of nutrients to regulate optimal food absorption [68, 69]. The CCK peptide with a sulfated tyrosine possesses high-affinity binding and full agonist activity to CCK1R. In the last three decades, enormous effort has been put forth to develop drugs to treat obesity and diabetes through the effects of CCK peptides and CCKAR [70]. However, none of the drugs have been approved for clinical use because some highly potent CCKAR agonists with a long duration of action, such as GW-5823, CE-326597, and Glaxo-11p, display adverse effects (diarrhea, nausea, and abdominal cramping) [70].

The octapeptide, CCK-8 (DYMGWMDF), which is derived from the C-terminal of CCK, is responsible for CCKAR activation, manipulating the appetite, and satiety [68]. The sulfated CCK-8 (DYSO3HMGMWDF-NH2)-bound CCKAR structures complexed with Gq, Gi, or Gs heterotrimeric, which were determined by cryo-EM technology, provide a structural basis for understanding the molecular details of ligand recognition and biased agonism during CCKAR activation [71]. By inspecting the orthosteric binding pocket of CCKAR occupied by sulfated CCK-8, the binding pocket was shown to be composed of TM3, 4, 5, 6, and 7 and ECL1, 2, and 3 (Figure 3). As shown in the binding pocket, the conformations of ECLs favor recognition of the N-terminal of CCK-8. The sulfate group of Y2 in CCK-8 forms a polar contact with R197ECL2, prompting the aromatic ring of Y2 in the CCK-8 hydrophobic bound with F185ECL2, M195ECL2, and K105ECL1. The CCK-8 residues, including M3, G4, and W5, interact with the interior surface of ECL3 via A343ECL3, L347ECL3, and S348ECL3. In addition, W5 and M6 in CCK-8 forms bifurcated hydrophobic cavities with the ECLs of CCKAR. For W5 in CCK-8, the side chain lies in the middle of the side chains of I352, and R336, making its indole nitrogen atom hydrogen bond with N333 and the carbonyl group of the main chain interacts with R336. M6 in CCK-8 occupies a shallow hydrophobic pocket, including F107ECL1, C196ECL2, T118ECL2, M121ECL3, and R336ECL2. At the bottom of the CCKAR binding pocket, the conformation of D7 in CCK-8 is stabilized by a polar interaction network with H210ECL2, N333ECL1, R336ECL1, and Y360ECL2. The aromatic ring of F8 in CCK-8 contacts Y176 through a polar hydrogen-π interaction.

The structural basis of the CCKAR binding pocket provides insight into the drug discovery process. One of the potential powerful tools is the use of a positive allosteric modulator (PAM), which displays no intrinsic agonist activity. The fully bioactive forms of CCK share the C-terminal amide and a sulfated tyrosine (sTyr) residue. Almost any reported mutagenesis or replacement of Gly-29, such as N-methylglycine or a fluorescent artificial amino acid, lead to major loss of CCKAR potency [72, 73]. Based on a systematic investigation of synthetic CCK-8 analogues with N-terminal linkage to fatty acids, peptide-based, long-acting, and stable highly selective CCKAR agonists have been identified and characterized [74]. By replacing Asp at the penultimate position of CCK-8 by DMetasp, novel agonists with high CCKAR selectivity have been achieved. In addition, inhibition of food intake in a pig model for up to 48 h after subcutaneous injection, as well as increasing plasma half-lives, were observed with an sTyr modification. Furthermore, compound NN9056, an N-terminal C18 fatty acid derivative with a free carboxyl group of CCK8, has potential for further development as an injectable anti-obesity drug [75, 76]. Moreover, when replacing the unstable methionine with two CCK-8 norleucine residues, the modified peptide displays maximum CCKAR selectivity, efficacy, and potency [74].

Individual CCKARs recognize and couple to divergent G-protein subtypes. Comparison of the three complexes formed by CCKAR and G-proteins suggest similar conformations. The most notable difference is at the tilts of the α5 helix of Gα subtypes (coupling ranking, Gq > Gi > Gs). The selectivity coupling between CCKAR and G-proteins is important for the physiologic response and development of therapeutic strategies for metabolic disorders.
Figure 3 | Cryo-EM structures of sulfated CCK-8 (DYSO3HMGMWDF-NH2)-bound CCKAR in complex with G proteins.

(A) Complex of CCKAR bound to sulfated CCK-8 coupled to Gi protein (PDB ID: 7EZH). (B) Complex of CCKAR bound to sulfated CCK-8 coupled to Gs protein (PDB ID: 7EZK). (C) Complex of CCKAR bound to sulfated CCK-8 coupled to Gq protein (PDB ID: 7EZM). The receptors, Ga\textsubscript{i}, Ga\textsubscript{s}, Ga\textsubscript{q}, G\textbeta{}, and G\gamma{}, are shown as ribbons colored in light blue, yellow, light green, light pink, marine blue, and magentas. The peptide ligand sulfated CCK-8 is displayed in orange stick. The detailed residues of the binding pocket of each complex coupled to the indicated G protein are shown in the right side of each panel.
5. PYY IN REGULATION OF SATIETY SIGNALS AND APPETITE EFFECTS

PYY belongs to the neuropeptide Y (NPY) family. PYY is secreted as PYY1-36 along with GLP1 from the intestinal L cells [77]. PYY1-36 is rapidly cleaved by dipeptidyl peptidase 4 (DPP4) to PYY3-36, which activates NPY receptor 2 (Y2R) and decreases food intake [78]. PYY3-36 signaling in food intake inhibition has provided insight for anti-obesity drug discovery, like developing PYY3-36 analogs [79]. In agreement with this notion, phase I trials have been carried out for two PYY3-36 analogs (NN9748 and NNC0165-1875) for long-acting activity in obesity therapy. Phase II trials are on-going using NNC0165-1875 combined with semaglutide for its treatment against obesity [8].

Structural understanding of ligand selectivity and biased signaling of Y2R is useful in the design of efficient and stabilized anti-obesity agents. Recently, the active state of Y2R bound to its endogenous ligands, including PYY3-36 and NPY, was resolved [80, 81]. In the structure of Y2R coupled with Gi protein, the agonist, NPY, adopts a hairpin shape with the unstructured N terminus and the \( \alpha \)-helix in the C-terminal region running antiparallel and occupying a large binding pocket involving ECLs and helices II–VII of Y2R (Figure 4). In appetite regulation, Y1R and Y2R display different responses and NPY-activated Y1R stimulates appetite, whereas NPY-activated Y2R suppresses food intake [82]. After cleavage of the two N-terminal amino acids, PYY3-36 preferentially activates Y2R but not Y1R [83]. Two Y2R cryo-EM structures bound to its endogenous ligands, NPY and PYY3-36, have been resolved and analyzed combined with molecular dynamics (MD) simulations and functional assays, elucidating Y2R activation and the molecular mechanism underlying exclusive binding of PYY3-36 to Y2R [81]. At the 6.58 position, the amino acid varies between Y1R and Y2R, which is F6.58 and V6.58, respectively. Another distinct feature of NPY/PYY3-36 recognition by Y2R occurs in the isoleucine-rich region of ECL2. Based on MD simulation analysis [81], the ECL2 of Y2R forms a relatively rigid hydrophobic cluster with PYY helix, but it is very flexible when simulated on Y1R. This finding may help explain the mechanism underlying the opposite physiologic response on appetite transduced by Y1R and Y2R activation.

6. APELIN ACTION IN ENERGY METABOLISM

Apelin is a peptide that is involved in glucose and lipid metabolism [84]. Adipose tissue is a possible source of plasma apelin because apelin was shown to be expressed and released by cultured adipocytes [85]. Apelin is an endogenous ligand of the apelin receptor (angiotensin receptor like-1, the angiotensin II protein J receptor, or APJR). APJR is a member of the class A \( \gamma \)-group of GPCRs which has diverse physiologic roles in angiogenesis, vasoconstriction, and energy metabolism [86]. To develop a novel agent beneficial in balancing energy metabolism, it is important to understand the fundamental biology of ligand recognition and receptor signal transduction. Fortunately, both crystal [86] and cryo-EM [29] structures of APJR have been resolved in recent years.
In the crystal structure of APJR bound to AMG3054, which is an apelin-17 mimetic peptide, a lactam constraining the curved two-site ligand binding mode was demonstrated. The C-terminal five amino acids of AMG3054 are inserted deeply into the orthosteric binding pocket nearly perpendicular to the membrane plane, which is termed “site 1.” In addition, the lactam ring formed by the side chain of K13 and E10 of the peptide made the main chain bend by approximately 90° from the membrane-perpendicular direction, termed “site 2” (Figure 5). In agreement with AMG3054 binding, mutations, including D172ECL2A, E174ECL2A, D184ECL2A, and E1945.31A, showed no effect on apelin-13 binding affinity [86]. In healthy humans, pyr-apelin-13 displayed a rapid dose-dependent increase in blood flow and reproducible effect after a saline washout. In contrast, apelin-36 showed a more durable response [87]. This observation suggested that additional interactions of longer peptides may display a prolonged duration of action.

7. MELANOCORTIN-4 RECEPTOR (MC4R) IN REGULATING ENERGY HOMEOSTASIS

MC4R is expressed in the paraventricular nucleus (PVN), which is a key component of the leptin-melanocortin pathway and responsible for the maintenance of long-term energy balance in humans, which provides a blueprint for the energetic state controlled by the central nervous system [88]. Two decades ago it was shown that MC4R-null mice exhibit late-onset obesity [89]. MC4R is activated by pro-opiomelanocortin (POMC)-derived neuropeptides α- and β-melanocyte-stimulating hormone (MSH). In parallel, the Agouti-related peptide (AgRP), which is inhibited by leptin, has been proposed as an inverse agonist of MC4R [90]. Greater than 80 pathogenic mutations naturally occurring in the leptin-melanocortin pathway have been shown to result in severe obesity in human and rodent models [91].

Unlike the direct interaction between ligands and their transmembrane bundles, the N-terminal domain of MC4R is responsible for ligand recognition and activation. The N-terminus of MC4R can act as a diffusible agonist; the minimal activation sequence is HLWNRS [90]. The first FDA-approved medication for obesity due to suspected leptin receptor (LEPR), POMC, or proprotein convertase subtilisin/kexin type 1 (PCSK1) deficiency is a cyclic peptide (setmelanotide) that displays high affinity towards MC4R compared to natural α-MSH [92]. The cryo-EM structure of MC4R-Gs-protein bound to setmelanotide has been resolved [93, 94]. Combined with the

Figure 5 | Crystal structure of APJR-AMG3054. The bound apelin-17 mimic peptide, AMG3054, adopted a curved binding conformation through a two-site binding mode. The C-terminal five amino acids of the peptide inserted into the binding cavity, which is approximately perpendicular to the membrane plane (termed “site 1”). The N-terminal half of the peptide bent by approximately 90 degrees binding to the surface grooves of APJR (termed “site 2”). The PDB ID of the analyzed structure is 5VBL.
Molecular structural data, further functional assay suggested that the allosteric interaction between the orthosteric binding pocket and G-protein binding sites is mediated by TM3 and facilitated by TM6.

Another study involving structural information of MC4R reported the complex with Gαs protein stimulated by α-MSH, selective ligand THIQ, and FDA-approved drugs (afamelanotide and bremelanotide), revealing the details of ligand recognition and receptor subtype selectivity [95]. Setmelanotide was shown to engage MC4R via hydrophobic interactions involving π-π stacking with F184, Y268, F284, and hydrogen bonding with S188 within the transmembrane regions (Figure 6A). Notably, one characteristic of setmelanotide binding to MC4R is a calcium ion coordinated by both the agonist and the receptor. The calcium ion was also observed in a similar position, while the antagonist SHU9119 (Figure 6B) binds to MC4R [94]. These structural studies provide insight into understanding the mechanism underlying MC4R activation and inhibition, setting the stage for development of new therapeutic agents to fight against obesity and other metabolic diseases.

In addition to stimulation of Gαs signaling, inducing anorexogenic signaling in the hypothalamus to result in negative energy balance, MC4R activation triggers signaling pathways involved in Gαi, Gαq, and β-arrestin recruitment [96, 97]. Both T1503.53 and H158ICL2 regulation of the MC4R signaling profile are likely related to G-protein selectivity [93]. To fully elucidate G-protein selectivity of MC4R, especially to differentiate Gq and Gi coupling, the structure of an agonist bound with MC4R-Gq complex will be necessary. The structural findings will help improve the development of novel anti-obesity drugs targeting the MC4R.

8. SIGNALING MECHANISM UNDERLYING CANNABINOID-1 RECEPTOR (CB1R) IN ANTI-OBESEITY TREATMENT

Obesity is related to an overactive endocannabinoid system. The human cannabinoid GPCRs, CB1 and CB2, have been the targets of intensive drug discovery efforts [98]. Progress in structure determination of cannabinoid receptors had identified the active, intermediate, and inactive conformations, providing valuable clues into signaling of cannabinoid receptors [99]. Diverse ligands can bind to the membrane-embedded orthosteric pocket or allosteric sites to modulate CB1R signaling via Gi/o or arrestin pathways. Understanding the bias signaling of CB1R may help decrease side effects in the therapeutic applications [100]. As the endocannabinoid system is involved in the regulation of energy metabolism, inverse agonists, such as rimonabant and tarabant, have been shown to be effective in the clinical treatment of obesity but failed to receive approval due to adverse CNS side effects [101]. Similarly, the CB1R inverse agonist, GFB-024, has been shown to mitigate diabetes-induced inflammation, displaying well-tolerated and no dose-limiting adverse effects [102]. Therefore, peripheral blockade of CB1R by inverse agonists avoiding CB1R binding in CNS might be an alternative strategy for obesity treatment.

Binding of an allosteric modulator is believed to induce a conformational change in the receptor that affects the potency or efficacy of an orthosteric agonist to generate receptor conformational states with unique structural and functional phenotypes. From the high-resolution (2.6 Å) crystal structure of the human CB1R (Figure 7A), the membrane-proximal N-terminal

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**Figure 6** | Structural models of MC4R bind to the agonist, setmelanotide, and the antagonist, SHU9119.

- **A** (Active conformation of MC4R binds to setmelanotide (PDB ID: 7PIU). Setmelanotide (yellow stick) engages MC4R (ribbon in light green) via hydrophobic interactions involving π-π stacking with F184, Y268, F284, and hydrogen bonding with S188. Ca2+ (green ball) is required for agonist binding.
- **B** (Inactive state of MC4R induced by SHU9119 (PDB ID: 6W25)). MC4R and SHU9119 are shown in blue ribbon and orange stick, respectively. The calcium ion (green ball) was also observed in a similar position as the antagonist, SHU9119.

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**Figure 7** | Different conformations of CB1R.

- **A** (Crystal structure of CB1R recognized by its antagonist, tarabant (PDB ID: 5U09). The inactive state of CB1R is shown in yellow ribbon. The antagonist, tarabant, is displayed in sticks and spheres with carbon in blue.
- **B** (Ternary structure of CB1R determined by X-ray crystallography (PDB ID: 6KQI). The active conformation of CB1R is represented as a pink ribbon. The agonist, CP55940, and the negative allosteric modulator, ORG27569, is shown in sticks and spheres with carbon in grey and orange, respectively.)
region of CB1R has been shown to form a critical part of the ligand-binding pocket, which is distinct from other lipid-activated GPCRs [98]. One of the first reported allosteric modulators is ORG27569, which is an indole-containing small molecule that increases binding of the full agonist, CP55940, in a concentration-dependent mode favoring an overall inactive state for G protein coupling, as revealed by spectroscopic studies [103, 104]. The crystal structure of CB1R with ORG27569 and the agonist, CP55940, elucidated ORG27569, a negative allosteric modulator (NAM) of CB1R, binds to the extra-helical site within the inner leaflet of the membrane overlying with a conserved site of cholesterol interaction in many GPCRs (Figure 7B).

Binding of ORG27569-CP55940 captures an intermediate state of CB1R, in which aromatic residues at the bottom of the orthosteric pocket adopt an inactive conformation, despite the large contraction of the binding pocket [100]. The reported crystallographic and cryo-EM structures of CB1R bound to the positive allosteric modulator (PAM), ZC2011, showed that the PAM binds to an extrahelical site in TM2-TM3-TM4 surface [105]. Recently, the complex of CB1R-Gi bound with an analog of endocannabinoid, AMG315, found “toggle switch” residues (F2003,36 and W3566,48) and the intracellular side of TM2 are a determinant of efficacy in Gi signaling [106]. These structural studies showed binding to TM2-TM3-TM4 and regulation of TM2 rearrangement is critical in allosteric modulations of CB1R, filling a gap in understanding CB1R allosteric and rational discovery of CB1R allosteric modulators.

While blockade of CB1R has been shown to halt diet-induced obesity [107], the solved structures of CB1R have advanced drug leads or rational design for obesity treatment. A series of 3,4-diarylpyrazolines bearing rational pharmacophoric pendants designed to limit brain blood barrier penetration were synthesized and displayed high binding affinity and potent CB1R antagonistic activities, acting as AMPK activators [108]. Other therapeutically promising ligands are biased agonists and biased allosteric modulators. The biased signaling of these drug leads can be measured or detected by receptor-G protein resonance energy transfer-based assays with fluorescence (FRET) and bioluminescence (BRET) biosensors, as well as cAMP, MAPK phosphorylation, and β-arrestin recruitment [110].

The dynamics of GPCRs enable diversity across ligand binding pockets and intracellular binding sites that increase the engaged pathways to balance efficacy and adverse drug reactions [111, 112]. As an important target in obesity and type 2 diabetes treatment, drugs that target GLP-1R, like semaglutide and liraglutide, are successful in regulating blood glucose levels but with adverse drug reactions ranging from nausea and diarrhea to pancreatitis [113]. It has been reported that GLP-1R activation engage 15 different pathways, including Gs, Gi1-4, G12-13, G11, G12, G13, Gαi, Gαβγ, Gαq, Gαβγ, GαGRK/Giβγ, and β-arrestin [114]. The agonists, GLP1(7-36), liraglutide, and exenatide, strongly activate Gi/0, while semaglutide and lixisenatide engage Gs, GRK/Giβγ, and β-arrestin pathways, which has been reported to have side effects, such as anxiety, nausea, and loss of appetite [115]. In addition, a small molecule agonist, danuglipron, displays biased engagement away from β-arrestin towards Gi and Gαi [114], with the side effects, including nausea, vomiting, and diarrhea [116]. Given the pleiotropic signaling of cardiovascular angiogenins II type 1 receptor (AT1R), it is a target for drug development for cardiovascular homeostasis. In the case of AT1R activation, both Ga and β-arrestin pathways need to be inactive to suppress aldosterone production to prevent chronic heart failure and hypertension [117]. Instead of focusing solely on Ga, β-arrestin-dependent signaling, other G protein types, like Gαq and G12/13, are also important in biased AT1R signal transduction [118].

The current molecular biology toolkit for GPCR has powered drug discovery towards the generation of several potent molecules for treatment of metabolic diseases [119, 120]. One of the major medical needs is the prevention or therapeutics for metabolic diseases, like obesity. It has been predicted that > 500 million individuals will be obese by 2035 [121]. To date > 30 GPCRs have been implicated in the development and progression of β-cell dysfunction, insulin resistance, TZDM, and obesity but only GLP1R has been successfully targeted in therapeutic strategies [122].

A lack of understanding of basic receptor pharmacology makes development of therapeutic drug leads immensely difficult [122]. Early stage target validation is required. Nonetheless, with the constant advances in technological platforms, including improved compound screening techniques, deorphanization strategies, novel targeting mechanisms, signaling bias, allostery, and the explosion in GPCR structural biology, combined with clinical data, we anticipate the medications will be aligned with the treatment paradigm in 10 years. Statistical significance is the historical benchmark to get
a medication approved. In the modern era, drugs need to show substantial benefit to the healthcare system to engender payment. Exploitation in bias signaling of GPCR structural biology provides the possibility and successfully targeting these GPCRs is even increasing. The current review highlights the mechanism on agonism or antagonism of these receptors, paving the way for basic pharmacologic probes which will enable these targets to fulfill the promise for metabolic disease therapeutics.

CONFLICTS OF INTEREST

No conflicts of interest, financial or otherwise, are declared by the authors.

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