THEMATIC REVIEW

RISING STARS

Targeting G protein-coupled receptors to regulate energy homeostasis

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Abstract

G protein-coupled receptors (GPCRs) have a critical role in energy homeostasis, contributing to food intake, energy expenditure and glycaemic control. Dysregulation of energy expenditure can lead to metabolic syndrome (abdominal obesity, elevated plasma triglyceride, LDL cholesterol and glucose, and high blood pressure), which is associated with an increased risk of developing obesity, diabetes mellitus, non-alcoholic fatty liver disease and cardiovascular complications. As the prevalence of these chronic diseases continues to rise worldwide, there is an increased need to understand the molecular mechanisms by which energy expenditure is regulated to facilitate the development of effective therapeutic strategies to treat and prevent these conditions. In recent years, drugs targeting GPCRs have been the focus of efforts to improve treatments for type-2 diabetes and obesity, with GLP-1R agonists a particular success. In this review, we focus on nine GPCRs with roles in energy homeostasis that are current and emerging targets to treat obesity and diabetes. We discuss findings from pre-clinical models and clinical trials of drugs targeting these receptors and challenges that must be overcome before these drugs can be routinely used in clinics. We also describe new insights into how these receptors signal, including how accessory proteins, biased signalling, and complex spatial signalling could provide unique opportunities to develop more efficacious therapies with fewer side effects. Finally, we describe how combined therapies, in which multiple GPCRs are targeted, may improve clinical outcomes and reduce off-target effects.

Key Words
- appetite
- combinatorial therapeutics
- diabetes
- G protein
- metabolism
- obesity

Invited Author’s profile

Caroline Gorvin is a Sir Henry Dale Fellow at the Institute of Metabolism and Systems Research (IMSR), University of Birmingham. She obtained her PhD in 2012 from the University of Oxford, where her research focussed on the cellular mechanisms by which mutations in a chloride-proton antiporter cause the renal disorder Dent’s disease. Caroline continued in Oxford undertaking postdoctoral research investigating the signalling and trafficking of the G protein-coupled receptor (GPCR), calcium-sensing receptor, and its role in calcium homeostasis. Caroline moved to the IMSR in 2018 to establish her research group investigating metabolic GPCRs. Her current research focusses on how metabolic GPCRs cross-talk and interact to regulate appetite and bone metabolism.
Introduction

G protein-coupled receptors (GPCRs) are the largest family of transmembrane proteins and are involved in the regulation of diverse physiological processes including regulation of the cardiovascular, nervous and endocrine systems. GPCRs have a critical role in energy homeostasis, which can be understood as the balance between food intake and energy expenditure and is required for the proper functioning of fundamental cellular processes including cell division, growth and repair, protein synthesis and immune responses. Dysregulation of energy expenditure can lead to metabolic syndrome (abdominal obesity, elevated plasma triglyceride, LDL cholesterol and glucose, and high blood pressure), which is associated with an increased risk of developing obesity, diabetes mellitus, non-alcoholic fatty liver disease and cardiovascular complications. These chronic diseases are increasing in prevalence and understanding the molecular mechanisms by which energy expenditure is regulated is vital for the development of effective therapeutic strategies to treat and prevent these conditions.

GPCRs are effective pharmacological targets as they are cell surface expressed and account for ~30% of the global market share of therapeutic drugs. In recent years, drugs targeting GPCRs have been the focus of efforts to improve treatments for type 2 diabetes and obesity, and increasingly targeting multiple GPCRs with physiological and/or pharmacological overlap has become the focus of efforts by the pharmaceutical industry. Moreover, as we have learned about the complexity of GPCR signalling, including modulation upon binding to interacting proteins, biased signalling and signalling from intracellular compartments (Fig. 1), new pathways have emerged that could provide unique opportunities to develop more efficacious therapies with fewer side effects.

In this review, we focus on nine GPCRs with roles in energy homeostasis (Table 1, Fig. 2) that are current...
Table 1  Overview of major GPCRs involved in energy homeostasis.

<table>
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<tr>
<th>Receptor</th>
<th>Major sites of expression/relevant activity</th>
<th>Endogenous ligand/s</th>
<th>Major signalling pathway</th>
<th>Physiological functions</th>
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<td><strong>Melanocortin axis receptors</strong></td>
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<tr>
<td>Melanocortin receptor 4 (MC4R)</td>
<td>Hypothalamus – PVN (Yeo et al. 2021)</td>
<td>αMSH, βMSH, γMSH (agonists) AgRP (antagonist)</td>
<td>Gs (major), Gq/11 Modified by MRAP2</td>
<td>Satiety</td>
<td>Setmelanotide for obesity Bremelanotide for use in women with low sexual desire</td>
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<td>Melanocortin receptor 3 (MC3R)</td>
<td>Hypothalamus – ARC (AgRP/POMC neurons) (Ghamari-Langroudi et al. 2018, Sweeney et al. 2021)</td>
<td>Gs</td>
<td>Gs</td>
<td>Bidirectional responses to calorie restriction and calorie intake, growth, onset of puberty</td>
<td>None*</td>
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<tr>
<td><strong>Growth hormone secretagogue receptor 1a (GHSR1a)</strong></td>
<td>Hypothalamus – ARC (NPY/AgRP neurons) (Müller et al. 2015), Vagal afferent neurons innervating GI tract (Davis et al. 2020), Pancreatic cells (Yin et al. 2020)</td>
<td>Ghrelin (agonist) LEAP2 (antagonist)</td>
<td>Gq Modified by MRAP2</td>
<td>Growth hormone secretion, appetite stimulation, insulin release, adiposity, gastric emptying</td>
<td>None*</td>
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<td><strong>Calcitonin family receptors</strong></td>
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<td>Amylin receptor (AMY_{1,2,3}R)</td>
<td>Hypothalamus, Brainstem (Zakariassen et al. 2020)</td>
<td>Amylin, CGRP</td>
<td>Gs/Gq</td>
<td>Glycaemic regulation, satiety, gastric emptying, Satiety, vasodilation, lipid homeostasis, glycaemic regulation</td>
<td>Pramlintide for use in type 1 &amp; type 2 diabetes</td>
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<td>Calcitonin gene-related peptide (CGRPR)</td>
<td>Adipose tissue (Makwana et al. 2021), Midbrain/pons parabrachial nucleus-amygdala/BNST, Peripheral somatosensory neurons (Campos et al. 2016)</td>
<td>CGRP</td>
<td>Gs/Gq</td>
<td>Satiety, vasodilation, lipid homeostasis, glycaemic regulation</td>
<td>Monoclonal antibodies and antagonists approved for migraine</td>
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<td><strong>Glucagon family receptors</strong></td>
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<td>Glucagon receptor (GCGR)</td>
<td>Liver (Geary et al. 1993), Kidney (Ouhilal et al. 2012), Hypothalamus – ARC (Quiñones et al. 2015)</td>
<td>Glucagon</td>
<td>Gs, Gq</td>
<td>Glycaemic regulation, may have some roles in satiety, lipid homeostasis, thermogenesis</td>
<td>None*</td>
</tr>
<tr>
<td>Glucagon-like peptide-1 receptor (GLP-1R)</td>
<td>Pancreatic β-cells (Portha et al. 2011, McLean et al. 2021), Vagal neurons innervating GI tract (Williams et al. 2016), Hypothalamus – ARC (He et al. 2019)</td>
<td>GLP-1</td>
<td>Gs</td>
<td>Glycaemic regulation, satiety</td>
<td>GLP-1R only: Exenatide, Liraglutide, Lixisenatide, Dulaglutide, Semaglutide, Combined GLP-1R &amp; GIPR: Tirzepatide</td>
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Melanocortin axis receptors

Melanocortin receptors

The melanocortin system communicates hormonal signals from peripheral tissues such as the pancreas and adipose tissue to the central nervous system and is responsible for regulating adrenal development and sexual behaviour, as well as energy homeostasis. The melanocortin peptide hormones (α, β and γ melanocyte-stimulating hormone (MSH) and adrenocorticotrophin hormone) are produced as post-translational products from the prohormone pro-opiomelanocortin (POMC) and act upon five melanocortin receptors (MC1R–MC5R), while two endogenous antagonists, agouti and agouti-related peptide (AgRP), suppress the receptors actions (Yeo et al. 2021). The MC3R and MC4R are the most important in energy expenditure. POMC is released from neurons of the hypothalamic arcuate nucleus (ARC) in response to hormonal signals such as leptin. The biologically active products αMSH and βMSH act on MC4R on the paraventricular nucleus (PVN) to increase cAMP signals and suppress food intake (Fig. 3). AgRP and neuropeptide Y (NPY)-expressing ARC neurons promote food intake by inhibiting MC4R and activating NPY-receptors on the PVN, respectively (Yeo et al. 2021).

NPY/AgRP neurons can also antagonise POMC neurons through γ-aminobutyric acid (GABA) signalling. Insulin and leptin suppress AgRP/NPY neurons, while ghrelin stimulates these neurons.

Human genetic studies have confirmed the importance of MC4R in the central regulation of energy homeostasis. Heterozygous loss-of-function mutations in MC4R are the most common monogenic form of obesity. Most inactivating MC4R mutations are missense and have poor cell-surface expression due to intracellular retention (Granell et al. 2010). Until recently, up to 25% of MC4R variants identified in obese cohorts were classified as non-pathogenic as they did not affect cAMP production. However, comprehensive in vitro investigation has since shown that these variants impair MC4R endocytosis, receptor dimerisation and/or G protein coupling (Brouwers et al. 2021). This indicates that targeting receptor dimerisation using bitopic ligands, or promoting G-protein coupling using allosteric modulators, could be effective treatments for early-onset obesity. Moreover, in vitro investigations have shown that many mutant MC4Rs and emerging targets to treat obesity and diabetes and describe how new insights into how these receptors signal could provide unique pharmacological targets for future therapies.
that are ER-retained do not impair receptor functionality, indicating that increased trafficking of these receptors to the cell surface by pharmacochaperones could be a viable treatment for hereditary obesity (Granell et al. 2010).

A subset of MC4R variants, identified in the UK Biobank, caused a gain in receptor function, and are associated with significantly lower BMI and lower odds of obesity, type-2 diabetes and coronary artery disease (Lotta et al. 2019). These variants increased receptor activity by biasing signalling towards β-arrestin recruitment and increased mitogen-activated protein kinase pathway signalling, indicating that drugs that preferentially activate these MC4R pathways may be effective in reducing weight and treating obesity-related disease (Lotta et al. 2019).

Melanocortin receptor signalling can be modulated by interactions with accessory proteins such as melanocortin-2 receptor accessory protein 2 (MRAP2), a heteromeric single-pass transmembrane protein, which enhances MC4R signalling. Global and Mc4r neuron-specific inactivation of MRAP2 increased food intake, weight gain and adiposity, and inactivating human MRAP2 variants have been identified in patients with hyperphagic obesity with hyperglycaemia and hypertension (Baron et al. 2019). Targeting MRAP2 could be effective in improving MC4R signalling, although the effect on other MRAP2-interacting GPCRs, including prokineticin and ghrelin receptors (Srisai et al. 2017) must be considered. Further studies to decipher how MRAP2 regulates different GPCRs, and how these interactions affect food intake and adiposity will help inform drug development strategies for MRAP2.

Efforts to target the melanocortin system have been hampered by adverse effects caused by a lack of melanocortin receptor selectivity or pathway specificity. Tachycardia and hypertension are of particular concern, as fusion of a highly-selective MC4R agonist led to increases in blood pressure and heart rate (Greenfield et al. 2009). However, studies of mice in which specific G proteins were deleted from the PVN showed that distinct pathways regulate MC4R-mediated effects on food intake and blood pressure. Thus, the ability of an MC4R agonist to inhibit food intake was lost in mice with PVN-deletion of Gq/11, while the agonist’s effect on blood pressure was lost in animals lacking Gs at the PVN (Li et al. 2016). As such, ligands that bias MC4R signalling to favour Gq/11 should suppress appetite while avoiding cardiovascular effects. Setmelanotide is the first approved anti-obesity drug for the treatment of rare genetic conditions associated with obesity and binds to MC4R with high affinity. Setmelanotide decreased food intake and adiposity, improved glucose tolerance and was associated with persistent weight loss in non-human primates, without effect on blood pressure and heart rate (Kievit et al. 2013). Moreover, setmelanotide treatment also increased weight loss in obese people with MC4R mutations (Collet et al. 2017). In vitro studies have indicated that setmelanotide is more efficacious in Gq/11-PLC activation than Gs-activation, which may explain why the drug is not

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**Figure 2**

Primary tissues and organs involved in energy homeostasis. Postprandial signals from the gut and vagal afferent neurons innervating the stomach, intestines, liver and pancreas relay signals to the brainstem to regulate whole-body functions. Changes in blood glucose levels are monitored by the pancreatic islet cells which function in a feedback loop regulated primarily by the insulin and glucagon hormones. Insulin and glucagon enter the brain from the circulation to activate hypothalamic neurons, which induces either anorexigenic or orexigenic effects. Peripheral adipose tissue thermogenesis and fat metabolism regulation are dependent on sympathetic outflow to it but can also signal back to the CNS or other organs through circulating leptin and adiponectin derived from adipose tissues to improve glycaemic regulation and overall energy homeostasis. The somatosensory system, primarily associated with temperature, haptic and pain sensations, may also provide information to the brain that inadvertently induces a response in energy homeostasis for example, cold-induced thermogenesis.
associated with adverse effects on blood pressure observed with other MC4R agonists (Heyder et al. 2021).

Another MC4R agonist, bremelanotide, which also binds to MC3R, has recently been approved in the United States for use in premenopausal women with low sexual desire. Data from two small randomised-control studies of obese premenopausal women showed that bremelanotide reduced caloric intake and weight loss, while having no adverse effects on blood pressure (Spana et al. 2022), indicating this may be another effective treatment for obesity. Antagonism of MC4R has also been investigated to treat the metabolic and dietary components of cachexia and anorexia that lead to tissue wasting. The peptide antagonist, TCMCB07, showed beneficial effects of increased food intake, body weight and preserved fat mass and lean mass in three rat models of cachexia and anorexia (Zhu et al. 2020). Moreover, another small molecule, PF-07258669, showed potent MC4R antagonism in an aged model of cachexia (Garnsey et al. 2023) and is currently being investigated in clinical trials.

Elucidating the physiological functions of MC3R has proven more difficult than that of MC4R. While early studies of MC3R in rodent models indicated roles for MC3R in energy metabolism, including in food anticipatory responses, and associations between MC3R deletion and mild late-onset obesity without hyperphagia or hypometabolism (Sutton et al. 2008), other studies produced seemingly contradictory findings. Studies in the past decade have shown that MC3R is required bidirectionally to control responses to both calorie restriction and calorie intake (Ghamari-Langroudi et al. 2018, Sweeney et al. 2021). Despite expression on both POMC and AgRP neurons, MC3R feeding effects are mediated predominantly by AgRP expression, supporting a model in which MC3R agonists increase food intake by stimulating GABA release from AgRP neurons, to suppress MC4R activity at the PVN (Ghamari-Langroudi et al. 2018, Sweeney et al. 2021).

Following the identification of inactivating MC4R mutations in obesity, several studies investigated genetic variants in MC3R in obese individuals, with inconsistent findings. A recent study investigating rare MC3R variants identified in the UK Biobank identified several loss-of-function MC3R variants that were associated with delayed puberty and reduced linear growth and lean mass (Lam et al. 2021). The authors suggested that MC3R may provide a link between nutritional status and linear growth and the onset of obesity.
Small molecules that act specifically on the MC3R have recently been described, with MC3R agonists shown to increase feeding and reduce anxiety, and MC3R-specific antagonists shown to suppress feeding in mice (Sweeney et al. 2021). This is consistent with studies showing MC3R-depletion enhances anorexia in mice (Sweeney et al. 2021) and indicates that modulating MC3R could be an effective therapy to increase weight gain and reduce anxiety in eating disorders while antagonising the receptor could promote weight loss. Additionally, MC3R agonists could be effective in patients with delayed puberty and/or short stature (Lam et al. 2021, Sweeney et al. 2021). Studies in humans examining the effects of these MC3R agonists and antagonists will be required to assess their effect on multiple parameters including weight, food intake, anxiety, growth, onset of puberty and cardiovascular functions (e.g. blood pressure and heart rate).

**Ghrelin receptor**

The growth hormone secretagouge receptor (GHSR1a) is a Gq-coupled receptor for the gut-derived hormone ghrelin, which has multiple roles in energy homeostasis including growth hormone secretion, stimulation of appetite, energy expenditure, insulin release, adiposity and gastric emptying (Müller et al. 2015). Within the hypothalamus, GHSR1a is expressed predominantly in NPY/AgRP neurons and ghrelin mediates its orexigenic effect by increasing NPY and AgRP expression and hyperpolarising POMC neurons, most likely mediated by the GABAergic NPY/AgRP neurons (Müller et al. 2015). Ghrelin and leptin have a reciprocal relationship aimed at increasing or decreasing adiposity. Fasting increases ghrelin while reducing leptin, and high leptin levels suppress gastric ghrelin secretion and prevent ghrelin-induced NPY neuron action (Kalra et al. 2005). GHSR1a is also expressed in several non-hypothalamic brain areas where it may function in taste sensation, reward behaviours and olfaction, all of which could contribute to ghrelin's effect on food intake (Müller et al. 2015).

As ghrelin secretion is increased during fasting and diminishes post-prandially in mice and humans, it was hypothesised that ghrelin may have a role in food anticipation and that blocking its actions may reduce food intake, adiposity and blood glucose (Mani et al. 2019). However, deletion of the GHSR or ghrelin gene from mice had little effect on body weight or insulin and leptin levels, although some differences were observed when mice were placed on a high-fat diet, likely due to ghrelin's role in fat utilisation (Müller et al. 2015). Moreover, ghrelin levels are low in obesity and binge eaters, while the orexigenic effect of ghrelin is less potent in obese mice, which indicated that a counter-regulatory mechanism may exist to limit ghrelin activity when food and nutrients are available (Müller et al. 2015, Mani et al. 2019). In 2018, an endogenous peptide, liver-expressed antimicrobial peptide 2 (LEAP2), which acts as an inverse agonist to GHSR1a constitutive activity and a competitive ghrelin antagonist, was discovered and appears to fulfill this counter-regulatory mechanism. LEAP2 is produced in the liver and small intestine and impairs ghrelin-induced food intake, growth hormone release and increases in blood glucose (Ge et al. 2018). LEAP2 is suppressed by fasting and is found at high levels in diet-induced obese mice and obese individuals, indicating that the plasma LEAP2/ghrelin ratio may be a key determinant in responses to feeding status, body mass and glucose (Mani et al. 2019). The discovery of LEAP2 has reinvigorated interest in targeting the GHSR1a to control appetite. Prior to this, GHSR1a antagonists, inverse agonists and antibodies designed to neutralise ghrelin had little long-term success in animal studies and clinical trials, with food intake quickly resuming in those studies in which an initial suppression of appetite was reported. However, a recent small clinical trial in 20 healthy individuals showed that intravenous administration of LEAP2 could successfully reduce food intake, meal duration and postprandial glucose, presenting the possibility that LEAP2 could be of clinical utility in type-2 diabetes and obesity (Hagemann et al. 2022). Further trials will be required to investigate the long-term effects of LEAP2 on food intake and glucose concentrations, and effects in patients with diabetes and obesity need to be investigated.

GHSR1a activity can also be modified by binding to accessory proteins such as MRAP2. Mice with targeted deletion of Mrap2 from AgRP neurons lose their ghrelin-induced increase in food intake (Srisai et al. 2017). *In vitro* studies showed that MRAP2 elicits signalling bias by enhancing Gq/11-mediated signalling, while inhibiting β-arrestin-mediated RhoA signalling and GHSR1a constitutive activity (Rouault et al. 2017, Srisai et al. 2017). This indicates that drugs that disrupt the GHSR1a and MRAP2 interaction sites may be more effective in reducing food intake than those targeting GHSR1a or MRAP2 alone. MRAP2 also regulates ghrelin-mediated reductions in insulin secretion by directly binding to GHSR1a in pancreatic δ-cells to facilitate ghrelin-induced somatostatin release that inhibits insulin release from pancreatic β-cells (Yin et al. 2020). Such ghrelin-mediated effects on insulin are protective mechanisms to prevent hypoglycaemia during fasting. Inhibition of GHSR1a...
with a selective antagonist has been shown to prevent ghrelin-mediated suppression of insulin release and improve glucose tolerance in obese mice (Esler et al. 2007). Therefore, GHSR1a antagonists or compounds that disrupt the GHSR1a–MRAP2 interaction site could be effective in promoting glucose-dependent insulin secretion in type-2 diabetics.

Finally, GHSR1a has been described to form heteromers with several GPCRs, which modify GHSR1a signalling. Although most interactions have been identified in cell lines and their physiological significance is unknown, interactions between GHSR1a and dopamine receptor-1 (DRD1) have been explored in detail in relevant tissues and animal models. GHSR1a enhances DRD1-induced initiation of hippocampal synaptic plasticity and formation of hippocampal memory, and these complexes are disrupted in Alzheimer’s disease (Tian et al. 2019). Targeting GHSR1a-DRD1 heteromers with a bitopic agonist could be an important preventative treatment for Alzheimer’s disease, although the effects on food intake and insulin secretion would need to be assessed carefully.

**Calcitonin family receptors**

The calcitonin family of peptides comprises calcitonin, amylin, α- and β-calcitonin gene-related peptide (CGRP) and adrenomedullin 1 and 2, which regulate diverse physiological functions including bone metabolism, appetite, nutrient utilisation and vasodilation (Hay et al. 2018). While calcitonin binds to the calcitonin receptor (CTR), the other peptides bind to distinct receptors consisting of a class B GPCR (CTR or CTR-like receptor (CLR)) heterodimerised with a receptor activity-modifying protein (RAMP), which is required for receptor trafficking to cell surfaces (Hay et al. 2018). Combination of the CTR with RAMP1, 2 or 3 produces AMY1, AMY2 and AMY3 receptors, respectively, which bind amylin, while CLR combined with RAMP1 forms the CGRP receptor, and CLR with RAMP2 and RAMP3, the receptors for adrenomedullin 1 and 2, respectively (Hay et al. 2018). Amylin and CGRP receptors are most relevant to energy metabolism.

**Amylin receptor**

Amylin is a pancreatic hormone that is synthesised and released alongside insulin from pancreatic β-cells and has roles in satiety, gastric emptying and glucagon secretion. Due to a paucity of highly selective antagonists to individual receptors and the ability of CGRP to bind to amylin receptors, it remains difficult to differentiate the effects of individual amylin receptors in vivo (Hay et al. 2018). A recent study would suggest that AMY1 receptor may be responsible for fat utilisation and storage, while AMY3 receptor is responsible for glycaemic regulation, although this study used RAMP knockout animal models, which would also eliminate CGRP receptor expression (Coester et al. 2020).

Amylin is released post-prandially and acts as a satiation signal binding to amylin receptors predominantly expressed in the area postrema and nucleus of the solitary tract (NTS) in the brainstem (Zakariassen et al. 2020). Amylin activity in the ventral tegmental area in the midbrain and nucleus accumbens in the forebrain may be linked to reducing rewarding effects from food. Amylin and leptin act synergistically, which may, in part, be due to amylin acting directly on ARC NPY/AgRP neurons co-expressing leptin receptor (Zakariassen et al. 2020). Amylin’s ability to slow post-prandial gastric emptying also contributes to satiety. Amylin also reduces the rate of glucose absorption and inhibits glucagon secretion (Gedulin et al. 2006).

Amylin’s beneficial effects on blood glucose and weight loss led to the development of a stable amylin analogue, pramlintide, approved for use in diabetes mellitus as an adjunct to insulin. When administered to people with obesity, pramlintide improved weight loss, while in patients with type-1 diabetes mellitus, it improved hyperglycaemia and delayed gastric emptying (Aronne et al. 2010). Pramlintide is generally well tolerated, although gastrointestinal side effects can occur, and its short half-life necessitates multiple daily injections. This led to the development of the long-acting amylin analogue cagrilintide. A recent clinical trial reported that once-weekly injection of cagrilintide significantly reduced body weight in people with a BMI >30 kg/m² (Lau et al. 2021).

**CGRP receptor**

CGRP is a product of alternative splicing of the calcitonin gene, expressed in the central and peripheral nervous systems and is a potent vasodilator. It is increased in headache disorders and its receptor is the target of monoclonal antibodies and antagonists for the treatment of migraine. Shortly after its discovery, CGRP was also reported to affect energy homeostasis with elevated levels reported in obese humans and animals (Liu et al. 2017b). However, deciphering CGRP function in food intake and appetite has proven difficult with animal models producing contradictory findings. Intraperitoneal administration of CGRP to mice significantly decreased food consumption and lowered energy expenditure (Sanford et al. 2019). However, CGRP knockout mice fed a high-fat diet have
significantly lower body weight, visceral fat and serum leptin, suggesting these animals are protected from diet-induced obesity (Liu et al. 2017b). Additionally, genetic ablation of heat-sensing CGRPα neurons promoted resistance to weight gain and increased energy expenditure by enhanced expression of fatty acid oxidation genes, higher ex vivo lipolysis in primary white adipocytes, and increased mitochondrial respiration from interscapular brown adipose tissue (ibAT) (Makwana et al. 2021). However, other animal models identified no changes upon CGRP deletion.

CGRP has been implicated in the control of meal termination, regulated by CGRP neurons that are directly innervated by AgRP-neurons (Campos et al. 2016). As CGRP regulates meal termination while other receptors regulate food intake (e.g. MC4R), it is feasible that targeting both pathways would have greater benefits than one pathway alone. However, a greater understanding of how CGRP signalling regulates vasodilation vs food intake will be required to avoid off-target effects (e.g. headaches) of potential CGRP agonists.

CGRP may also regulate glycaemic control. CGRP reduced glucose-stimulated insulin secretion and increased glucagon in animals (Nilsson et al. 2016), while deletion of the CGRP receptor in mice improved glucose tolerance and insulin sensitivity (Liu et al. 2017b). However, other studies were unable to confirm these effects on insulin (Sanford et al. 2019). Differences in findings could be explained by some studies using supraphysiological concentrations of CGRP, or due to the effects of amylin, which can also bind to CGRP receptors. More promisingly, a monoclonal antibody against CGRP was recently shown to lower hyperglycaemia and adiposity in a type-2 diabetes mouse model, suggesting targeting CGRP could have therapeutic benefits in diabetes (Halloran et al. 2020).

**Glucagon family receptors**

**Glucagon receptor**

The glucagon receptor (GCGR) binds the hormone glucagon, secreted by pancreatic α-cells, which increases glucose and fatty acid concentrations. GCGR activity stimulates glucose production by increasing hepatic gluconeogenesis and glycogenolysis (Janah et al. 2019). Due to these actions on insulin and glucose, suppression of glucagon action was initially considered a good strategy to treat diabetes. However, animal studies have shown that hypothalamic glucagon signalling inhibits hepatic glucose production and suggested that hypothalamic glucagon resistance disrupts glucose homeostasis in diabetes and obesity (Mighiu et al. 2013). Moreover, glucagon stimulates energy expenditure, reduces food intake and enhances weight loss in rodent and human studies, which suggests glucagon agonism would be beneficial in obesity.

The mechanism by which glucagon and GCGR influence weight loss is incompletely understood. There is some evidence that in BAT, GCGR promotes thermogenesis through stimulation of lipid oxidation and free fatty acid synthesis, expression of thermogenic genes, increased body core temperature and oxygen consumption (Townsend et al. 2019), while GCGR expressed in white adipose tissue (WAT) can also lead to the ‘browning’ of WAT by expression of thermogenic genes (Townsend et al. 2019). However, a recent mouse model in which Gcgr was deleted from WAT showed no effects on body weight or glucose homeostasis and that physiological levels of glucagon did not regulate lipolysis (Vasileva et al. 2022). Moreover, a recent study using mice with deletion of Gcgr from BAT showed the receptor was not required for control of body weight, adiposity, core temperature or energy expenditure (Beaudry et al. 2019). Liver-derived FGF21, which is increased by acute and chronic GCGR activation may contribute to glucagon-mediated weight loss. Global deletion of FGF21 or neuronal deletion of its obligate co-receptor β-klotho from mouse partially impaired the effect of a GCGR agonist on weight loss demonstrating a central regulation of GCGR-mediated weight loss (Nason et al. 2021). It is likely that other mechanisms also contribute to glucagon-mediated weight loss.

Glucagon receptor activity also induces satiety and reduces meal size (Geary et al. 1993). In the liver, GCGR activity results in a satiety signal via the vagal afferent nerves to the NTS neuronal region in the brainstem, and subsequently a neuronal signal to the hypothalamus (Fig. 3) (Geary et al. 1993). Glucagon can also diffuse through the blood–brain barrier and bind directly to the GCGR expressed in the hypothalamic ARC (Quiñones et al. 2015). Hypothalamic GCGR activity inhibits AgRP-expressing neuron activity, thereby attenuating orexigenic effects, whereas central resistance to glucagon-induced hypophagia contributes to the development of obesity (Quiñones et al. 2015). However, further work is needed to properly differentiate the pharmacology of central GCGR and the closely-related glucagon-like peptide-1 receptor.

**GLP-1 receptor**

Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinoctropic polypeptide (also known as gastric
GLP-1R agonists and GLP-2. The receptors for GLP-1 and GLP (GLP-1R and GIPR, respectively) are class B GPCRs with large extracellular domains (ECD), and the peptides bind in a two-step mechanism to both the ECD and the transmembrane domain. GLP-1R and GIPR are highly expressed in pancreatic β-cells where they promote glucose-stimulated insulin secretion (McLean et al. 2021). GLP-1 also slows gastric emptying and increases satiety.

GLP-1R activates cAMP pathways including PKA and EPAC2 to stimulate insulin secretory granule exocytosis from pancreatic β-cells. GLP-1R activation promotes proliferation and suppresses apoptosis of β-cells, thereby preserving β-cell mass, which could further improve glycaemic control (Portha et al. 2011). GLP-1 can suppress glucagon secretion, although the mechanism and whether GLP-1R is involved remain unresolved (McLean et al. 2021).

The desirable effects of GLP-1 on glycaemic control led to the development of a series of GLP-1 mimetics with a longer half-life than natural GLP-1 that bind and activate GLP-1R for use in the treatment of diabetes. A series of GLP-1R agonists with resistance to dipeptidyl dipeptidase-IV breakdown are used routinely to treat type-2 diabetes (Table 1) and can be administered daily to weekly (Khoo & Tan 2020). Liraglutide and semaglutide were subsequently approved for managing weight loss in obesity when appreciable losses of weight were observed in patients with type-2 diabetes (Khoo & Tan 2020). GLP-1R agonists have also been shown to improve cardiovascular complications such as stroke, myocardial infarction and cardiovascular death and are approved for use in type-2 diabetics at high risk of cardiovascular disease (Khoo & Tan 2020). Beyond metabolic disease, GLP-1R agonists have been investigated in a range of tissues and diseases including, Alzheimer’s disease, Parkinson’s disease, adrenal function, opioid dependency, bone turnover, angiogenesis and chronic pain. A detailed review of these studies is beyond the scope of this review and interested readers are referred to a recent publication detailing many of these (Zhao et al. 2021).

GLP-1R agonists are associated with gastrointestinal side effects at high doses, which limits their effectiveness in weight loss and reduces patient compliance. Therefore, improved therapies will be required to target GLP-1R while reducing these off-target effects. Current strategies include designing novel GLP-1R peptides with improved efficacy or biased agonism to reduce side effects, or combination therapies targeting GLP-1 and other gut hormones. Peptides with modification of the N-terminus of exendin-4 produce agonists with distinct effects on receptor trafficking (Jones et al. 2018). Agonists that reduced β-arrestin recruitment, receptor internalisation and increased receptor recycling improved insulin release in human islets and glucose control in mice. However, these agonists had no effect on food intake (Jones et al. 2018). Another modified agonist (GL0034 based on semaglutide) which had enhanced cAMP potency had similar effects on GLP-1R mediated insulin secretion and weight loss to semaglutide (Jones et al. 2022). This indicates that there may be distinct signalling and trafficking pathways involved in GLP-1R-mediated glycaemic control vs appetite suppression. Subsequent studies showed that activated GLP-1R is recruited to membrane nanodomains in a mechanism that involves palmitoylation, and this clustering drives GLP-1R signalling responses and internalisation (Buenaventura et al. 2019). Further studies of agonists that selectively modify these distinct signaling pathways could improve GLP-1R targeting therapies for diabetes and obesity. A recent study has proposed that inhibition of the K\textsubscript{ATP} channels by mutation or long-term use of sulfonylureas leads to persistently depolarised β-cells in diabetes and that GLP-1R switches coupling from Gs-to-Gq signalling to amplify insulin secretion (Oduori et al. 2020). Whether GLP-1R agonists can be modified to preferentially activate the Gq pathway and what the physiological consequences are remain to be explored.

The mechanisms by which GLP-1 reduces food intake and promotes weight loss are incompletely understood and probably involve several mechanisms. Some vagal afferents express GLP1R and it was proposed that the effects of GLP-1R agonism on food intake could contribute to the vagal detection of intestinal nutrients. However, animal studies have shown that GLP-1R-expressing neurons in the stomach and the intestines are primarily mechanosensitive, which likely respond to GI distention rather than intestinal GLP-1 activating GLP-1R (Williams et al. 2016). As synthetic GLP-1R agonists can penetrate the blood–brain barrier, it is likely that their effects on food intake are driven by direct effects on GLP-1R in the brain. GLP-1R is expressed throughout the CNS and several pathways involving GLP-1R-positive neurons in the hindbrain and hypothalamus have been described to regulate food intake. Studies in mice showed that stimulation of GLP-1R-expressing NTS neurons that project to the PVN is sufficient to mediate food intake

[Note: The document contains references and links to further reading.]

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and maintain body weight. Using chemogenetics and slice physiology, GLP-1R in the PVN was shown to augment corticotropin-releasing hormone neuronal excitatory synaptic strength via a mechanism involving PKA-driven enhancement of AMPA receptor membrane trafficking (Liu et al. 2017a). NTS neurons expressing pre-proglucagon (PPG) also project to the stria terminalis, an area associated with feeding, stress and reward behaviour (Williams et al. 2018).

A study using fluorescently labelled liraglutide in mice revealed the drug is targeted to POMC neurons and that it indirectly inhibits NPY/AgRP via a GABA-dependent signalling mechanism (Secher et al. 2014). A subsequent study that used neuron-specific transgenic mice showed that liraglutide directly activated ARC POMC neurons via TRPC5 calcium channels. GLP-1R was also shown to activate a signalling pathway involving TRPC5 and K<sub>ATP</sub> channels to enhance the activity of GABAergic neurons, which inhibit NPY/AgRP neurons via post-synaptic GABA-A receptors (He et al. 2019). Combined activation of POMC neurons with liraglutide and leptin led to enhanced activation of the TRPC5 pathway indicating that therapies combining agonism of the two receptors may be beneficial in suppressing food intake (He et al. 2019). This co-activation probably involves independent activation rather than co-stimulation of signalling pathways as subsequent mouse models showed that leptin receptor and GLP-1R POMC neurons are largely non-overlapping (Biglari et al. 2021).

**GIP receptor**

Like GLP-1R, GIPR is highly expressed in pancreatic β-cells, increases cAMP-PKA signalling to enhance insulin secretion and suppresses apoptosis which may improve β-cell health (Widenmaier et al. 2010). GIP has a bifunctional role in regulating blood glucose. In healthy subjects, GIP has no effect on glucagon responses during hyperglycaemia, whereas GIP increases glucagon and has no effect on insulin secretion during fasting and hypoglycaemia (Tan et al. 2013). GIPR also regulates adipocyte function, although findings are contradictory. Mice with deletion of the GIPR are resistant to diet-induced obesity, although chronically elevating GIP has been shown to reduce DIO. Moreover, GIP has been shown to have no effect on weight loss or on energy expenditure in overweight and obese subjects (Finan et al. 2013). Differences in the efficacy and pharmacokinetics of agonists and antagonists used in the studies, species differences or GIPR desensitisation could explain some of these discrepancies. In favour of the latter argument, lowering circulating glucose, which is known to promote GIPR desensitisation, improves the insulin response of type-2 diabetic patients treated with GIP and GLP-1, indicating that co-agonism may enhance GLP-1 agonist therapies.

GIPR is expressed at multiple sites within the brain including the ARC, PVN, dorsomedial hypothalamic nucleus (DMH), NTS and area postrema (Adriaenssens et al. 2019, Borner et al. 2021). Activation of hypothalamic neurons in mice using designer receptors exclusively activated by designer drugs (DREADDs) suppressed food intake (Adriaenssens et al. 2019). Co-activation of neurons that co-express Gipr and Glp1r did not have any further effect on acute food intake than activating Glp1r neurons alone (Adriaenssens et al. 2019). Subsequent studies have shown that CNS-specific knockout of GIPR lowers body weight and improves glucose metabolism and that administration of fatty acyl-GIP induced acute neural activation in key feeding centres of the hypothalamus including ARC, DMH, ventromedial hypothalamus and the lateral hypothalamus providing further evidence that GIP contributes to the central control of energy homeostasis (Zhang et al. 2021). Additionally, studies in rodents and shrews have indicated that GIPR in the hindbrain may have an anti-emetic role, although it is unknown whether these neurons contribute to other metabolic functions (Borner et al. 2021).

**5-hydroxytryptamine receptor 2C**

Serotonin (5-hydroxytryptamine, 5-HT) within the brain has been known to regulate appetite and body weight for more than 40 years. Compounds that stimulate 5-HT, including D-fenfluramine and sibutramine, were amongst the earliest treatments for obesity; however, they were withdrawn from clinical use due to their side effects including increased heart rate and blood pressure (D’Agostino et al. 2018). Mouse knockout studies demonstrated that the GPCR, 5-hydroxytryptamine receptor 2C (5-HT<sub>2C</sub>R), is responsible for the 5-HT effects on feeding, which led to the development of the selective 5-HT<sub>2C</sub>R agonist lorcaserin. Although lorcaserin was clinically approved to improve weight loss, it was withdrawn in 2020 due to a possible increased occurrence of cancer. An improved understanding of how lorcaserin produces its therapeutic effect, and how 5-HT<sub>2C</sub>R controls food intake, could improve future drug design targeting this receptor and improve off-target effects (Wagner et al. 2022).
Studies using DREADDs, conditional knockout and reporter mice revealed 5-HT_{2C}Rs at the NTS and the ARC are required for 5-HT effects on appetite and food intake and that lorcaserin induces its appetite-suppressive effects by acting upon POMC neurons within these regions (D’Agostino et al. 2018). Recently, lorcaserin was shown to also activate PPG NTS neuron populations to reduce food intake (Wagner et al. 2022). Using specific agonists of MC4R and GLP-1R, the PPG-NTS and POMC-NTS/ARC neuronal pathways were shown to be functionally independent targets of lorcaserin, indicating that targeting GLP-1R and 5-HT_{2C}R may be beneficial. Indeed, combining lorcaserin with liraglutide or exendin-4 produced greater reductions in food intake in mice (Wagner et al. 2022), suggesting drugs targeting these two receptors may produce greater effects in obesity. Moreover, as the GLP-1R and MC4R neuronal pathways were shown to be independent, some patients with MC4R mutations may benefit more from GLP-1R agonists than setmelanotide or lorcaserin (Wagner et al. 2022).

**Combinatorial GPCR-targeted therapeutics**

Observation of improved responses to GLP-1 in combination with other GPCR agonists in animal models led to the development of several co-agonists. As GLP-1 and glucagon have high sequence homology, researchers have designed unimolecular receptor co-agonists that should bind to both receptors. Investigators have designed several such compounds with different ratios of GLP-1 to glucagon. One compound that targets GLP-1R and GCGR, NN-1177, was terminated after the phase 1 clinical trial due to adverse effects including increased pulse rates (Coskun et al. 2022). Similar findings were observed for other compounds targeting both receptors (e.g. cotadutide and mazdutide), although when administered in multiple ascending doses, elevations of pulse rate decreased over time (Coskun et al. 2022, Ji et al. 2022).

Early studies showed that co-agonism using GIP and GLP-1 improved glucose-stimulated insulin release and adiposity in rodents and substantially reduced GI side effects in humans (Finan et al. 2013). Tirzepatide, a unimolecular GIP and GLP-1 agonist with greater potency for GIPR than GLP-1R, had enhanced effects on glycaemic control and weight loss than a GLP-1R agonist in type-2 diabetic patients (Khoo & Tan 2020). Once-weekly tirzepatide was also recently shown to improve weight loss in obese patients. However, adverse GI effects were reported in diabetic and obese patients indicating that favouring GIP agonism does not remove these adverse effects (Jastreboff et al. 2022).

In parallel with studies investigating GIP and GLP-1 dual agonists, other researchers have developed drugs that inhibit GIPR activity, while activating GLP-1R. AMG-133 is a GIPR-inhibiting antibody conjugated to a GLP-1R agonist, which acts by promoting receptor internalisation to enhance sustained endosomal cAMP responses, resulting in reduced body weight in obese animals (Lu et al. 2021). AMG-133 has undergone phase 1 clinical trials, and Amgen press releases have reported up to a 14.5% reduction in body weight in individuals with obesity without diabetes. Similar to other studies, AMG-133 was associated with adverse GI effects; however, the once-monthly administration of AMG-133 may prove advantageous when compared to tirzepatide that requires weekly doses.

A tri-agonist unimolecular peptide, LY3437943, with balanced GLP-1R and GCGR activity, but more GIPR activity, decreased body weight and improved glycaemic control in obese mice. In a first in-human study, LY3437943 achieved similar weight loss effects to tirzepatide and had a substantial effect on appetite control, as well as reductions in fasting triglycerides. Adverse effects including pulse rate were not significantly different to the placebo group (Coskun et al. 2022).

Several studies have investigated the utility of combined therapies targeting amylin receptor with other GPCRs, including with GLP-1R. In phase 1 clinical trial, individuals with a BMI > 27 kg/m² were administered once-weekly cagrilinitide in combination with semaglutide subcutaneously. When compared to semaglutide alone, patients receiving both cagrilinitide and semaglutide had greater weight loss, improved glycaemic control and no differences in adverse effects observed (Enebo et al. 2021). Further studies will be required in larger cohorts, but this early study indicates that combining drugs targeting amylin receptors and incretin receptors could be beneficial in patients with diabetes and obesity. This is an area of research that is likely to continue to develop as Novo-Nordisk recently announced that its amylin receptor and GLP-1R dual-agonist, Amycretin, is set to enter phase 1 clinical trials in individuals living with obesity. Co-administration of amylin and an MC4R agonist to rats had a greater effect on weight loss and food intake than treatment with amylin alone (Roth et al. 2012), and amylin-induced stimulation of thermogenesis in iBAT was blocked by an MC4R antagonist (Li et al. 2019), indicating that combined treatment may be beneficial in obesity.
As stated, amylin and CGRP have some cross-affinity at their respective receptors, complicating the deciphering of their respective roles in energy homeostasis. However, their shared effects on glycaemic control and adiposity led to the development of dual amylin-calcitonin receptor agonists (DACRA) that target both receptors with superior potency. The first generation of DACRAs had modest effects on food intake and body weight and improved glycaemic regulation but required frequent (daily) dosing. Recently a long-lasting DACRA, KBP-066A, that can be administered once weekly was described. In rats, KBP-066A improved fasting blood glucose, plasma insulin and oral glucose tolerance and reduced body weight in obese diabetic rats (Andreassen et al. 2021), indicating that a regime that is more likely to be tolerated by patients still has a beneficial effect on energy homeostasis. Encouragingly, combined treatment of high-fat diet-fed rats with a short-acting DACRA (KBP-089) and the GLP1R agonist liraglutide had an additive effect, lowering body weight, reducing fat depot sizes, improving glucose metabolism and gastric emptying (Larsen et al. 2021).

Conclusions

An increased understanding of how appetite is controlled, and the GPCRs that regulate these physiological pathways, has led to a promising number of targets for the treatment of diabetes and obesity. Drugs that target several of these GPCRs have already been approved, while many more are in development or have been tested in pre-clinical models and clinical trials. However, the deletion of GPCR genes in animal models and agonism/antagonism of individual receptors have demonstrated that many hormonal pathways converge to regulate appetite and the complexities of these pathways remain incompletely understood. Targeting multiple GPCRs simultaneously may improve treatments, and early reports of combination drugs targeting GCGR/GLP-1R/GIPR show promising additive improvements in weight loss. Additionally, the unimolecular nature of many of these compounds, rather than multiple individual GPCR-targeting drugs may improve patient compliance. It is likely many more combinatorial drug strategies will emerge in the coming years.

Despite promising developments, many GPCR-targeting drugs for the treatment of diabetes and obesity are associated with side effects and these may reduce patient compliance. More research is required to understand how different GPCR signalling pathways regulate functions in individual tissues. An increased understanding of these nuanced differences in signalling could inform the design of biased ligands that reduce or remove off-target effects. Additionally, large-scale genetics projects investigating rare coding variants associated with obesity have yielded new GPCR targets, while GPCRs in peripheral tissues involved in energy homeostasis (e.g. free fatty acid receptors in adipose tissue) could emerge as new targets for the next generation of drugs for metabolic disease. There is still clearly much work to do in this area.

Declaration of interest
The authors have no conflicts of interest to declare.

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References


Deeley RJ, O'Malley ME, Bolger AN, Cameron GS, Ainslie PN, Doherty TJ & Elks CR 2017 Insights into the pharmacodynamic and pharmacokinetic properties of the dual incretins maximize metabolic benefits in rodents, monkeys, and humans. Nature Medicine 23 79–86. (https://doi.org/10.1038/nm.4174)


Granell S, Mohammad S, Ramanagoudr-Bhojappa R & Baldini G 2010 Obesity-linked variants of melanocortin-4 receptor are misfolded in the endoplasmic reticulum and can be rescued to the cell surface by a chemical chaperone. Molecular Endocrinology 24 1805–1821. (https://doi.org/10.1210/me.2010-0071)


Williams EK, Chang RB, Strohlic DE, Umans BD, Lowell BB & Liberles SD 2016 Sensory neurons that detect stretch and nutrients in the digestive system. *Cell* **166** 209–221. (https://doi.org/10.1016/j.cell.2016.05.011)


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