Emerging combinatorial hormone therapies for the treatment of obesity and T2DM

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Abstract | Peptide hormones and proteins control body weight and glucose homeostasis by engaging peripheral and central metabolic signalling pathways responsible for the maintenance of body weight and euglycaemia. The development of obesity, often in association with type 2 diabetes mellitus (T2DM), reflects a dysregulation of metabolic, anorectic and orexigenic pathways in multiple organs. Notably, therapeutic attempts to normalize body weight and glycaemia with single agents alone have generally been disappointing. The success of bariatric surgery, together with emerging data from preclinical studies, illustrates the rationale and feasibility of using two or more agonists, or single co-agonists, for the treatment of obesity and T2DM. Here, we review advances in the science of co-agonist therapy, and highlight promising areas and challenges in co-agonist development. We describe mechanisms of action for combinations of glucagon-like peptide 1, glucagon, gastric inhibitory polypeptide, gastrin, islet amyloid polypeptide and leptin, which enhance weight loss whilst preserving glucoregulatory efficacy in experimental models of obesity and T2DM. Although substantial progress has been achieved in preclinical studies, the putative success and safety of co-agonist therapy for the treatment of patients with obesity and T2DM remains uncertain and requires extensive additional clinical validation.

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Introduction

With levels of type 2 diabetes mellitus (T2DM) and obesity reaching epidemic proportions in many countries, the need to find safe and effective therapies for the treatment of these disorders is increasing. Energy intake and expenditure are carefully balanced to maintain body weight by an active complex process that depends on neuronal networks in the brain, which integrate afferent inputs from multiple peripheral organs, such as the pancreas, the gut and adipose tissue, via orexigenic and anorectic hormones. Although obesity is associated with resistance to adipokines, such as leptin, that enhance energy expenditure and reduce food intake, this resistance is partially reversible with weight loss or pharmacotherapy in preclinical studies. Bariatric surgery, including gastric bypass, produces substantial and durable weight loss that seems sustainable over time in most patients. In long-term studies, these procedures are associated with reduced mortality.[1] These positive results have not been replicated by any pharmacologic therapies to date. Nonetheless, recognition is growing that some of the benefits attributed to bariatric procedures reflect changes in the circulating levels of gut peptides that are implicated in the central control of satiety and body weight, including glucagon-like peptide-1 (GLP-1), peptide YY (PYY) and oxyntomodulin (Box 1).

Classic concepts of gut hormone action focus on the postprandial state: enhanced secretion of gut peptides reduces gut motility, decreases appetite and facilitates nutrient disposal. However, the use of receptor antagonists and knockout mice has revealed that some gut hormones also exert physiologically important actions when present at low levels in the interprandial and fasting states.[2] For example, infusion of GLP-1 receptor (GLP-1R) antagonists increases fasting glucagon and glucose levels in multiple species, and mice genetically modified to lack the Glp-1 receptor (*Glp1r*$^{-/-}$) exhibit significantly increased levels of fasting glucose.[3] Conversely, activation of GLP-1R signalling in the fasted-overnight state reduces glucagon and decreases fasting glucose levels in rodents and humans, contributing an important component of its therapeutic glucose-lowering effect.[2] These findings provide a rationale for the pharmacological activation of gut hormone pathways during both the postprandial and fasting states to optimize control of glucose homeostasis and body weight.

Efforts to use single molecules that target one pathway for the treatment of obesity have been largely unsuccessful or suboptimal. For example, although injectable GLP-1R agonists have produced substantial weight loss in a small subset of obese patients with and without T2DM,[4] the weight loss achieved with a gastric bypass is substantially greater. Evidently, compensatory mechanisms successfully 'protect' against weight loss induced by a single agonist, which might reflect the evolutionary

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Key points

- Multiple enteroendocrine and adipose-derived hormones convey satiety signals to the central nervous system
- Many regulatory peptides exert glucoregulatory actions independent of their effects on appetite and body weight
- Most organisms exhibit robust counter-regulatory responses to one anorectic agent acting through a single signalling pathway
- Combining two or more hormones with complementary anorectic and glucoregulatory activities might be a promising approach for the treatment of obesity and diabetes mellitus
- The safety of new co-agonists in the brain and cardiovascular system, and their potential for promoting cell proliferation, requires careful scrutiny

Box 1 | Endocrine hormones currently used for co-agonist therapies

- Glucagon is a 29 amino acid proglucagon-derived peptide that is synthesized in and secreted from pancreatic islet α cells. Glucagon is also produced in the brain, predominantly in the brainstem, from where it is transported to distant regions of the CNS, including the hypothalamus.[76]
- GLP-1, a 30 amino acid proglucagon-derived peptide, is continuously secreted at low levels from L cells of the small and large intestine in the fasting state, and plasma levels of GLP-1 rise rapidly in response to nutrient ingestion.
- Oxyntomodulin is a 37 amino acid proglucagon-derived peptide that is secreted together with GLP-1, GLP-2 and glicentin from enteroendocrine L cells in proportion to nutrient ingestion. Oxyntomodulin is also produced in the brain, predominantly in the brainstem, and transported to distal regions of the CNS, such as the hypothalamus.[76,77]
- GIP, which is also known as glucose-dependent insulinotropic polypeptide, is a 42 amino acid incretin hormone secreted continuously at low basal levels from enteroendocrine K cells, which are predominantly localized to the proximal small bowel. GIP secretion is rapidly stimulated by nutrient intake.
- Gastrin, a 34 amino acid peptide derived from preprogastrin, is secreted by the enteroendocrine G cells in the antrum of the stomach in response to a meal. Gastrin circulates in various forms (gastrin 17 and gastrin 34) and exerts metabolic actions via CCK receptors type A and B.
- IAPP, which is also known as amylin, is a 37 amino acid peptide synthesized in pancreatic β cells that is co-secreted with insulin in response to nutrient intake. IAPP is also produced in enteroendocrine cells and enteric neurons within the gastrointestinal tract.
- Leptin is a 146 amino acid protein secreted from adipocytes, and circulating leptin levels are proportional to the extent of adipose tissue mass.
- PYY is a 36 amino acid member of the neuropeptide Y family that is released from enteroendocrine cells in the ileum and colon in response to nutrients, particularly fat.

Abbreviations: CCK, cholecystokinin; CNS, central nervous system; GIP, gastric inhibitory polypeptide; GLP, glucagon-like peptide; IAPP, islet amyloid polypeptide; PYY, peptide YY.

importance of energy conservation for the preservation of reproductive fitness and long-term survival. Hence, multiple redundant pathways could have developed that promote food ingestion, maintain body mass and resist long-term reduction of body weight. The available evidence supports the concept that targeting two or more anorectic pathways might be necessary to effectively achieve substantial weight loss. Indeed, FDA approval of the combination therapy phentermine and topiramate for the treatment of obesity highlights the growing recognition that co-agonists can produce substantially greater metabolic benefits than single agents.[5] This Review highlights preclinical data, mechanisms of action, clinical advances and safety considerations of co-agonists and combinatorial peptide approaches for the treatment of obesity and T2DM.

Glucagon

Classic actions of glucagon encompass the control of hepatic glucose production as a defence against hypoglycaemia via stimulation of a single glucagon receptor (GCGR). Dysregulation of glucagon secretion occurs early on in the pathogenesis of glucose intolerance and T2DM and contributes to the development of hyperglycaemia.[6]

Considerable experimental evidence from preclinical studies supports a role for glucagon as a satiety factor. Pharmacological infusion of glucagon increases oxygen consumption in rats, in part through effects on brown adipose tissue (BAT),[7] and glucagon infusion in humans increases resting energy expenditure under experimental conditions that are associated with low levels of circulating insulin, for example, after somatostatin infusion.[8] Short-term glucagon infusion also increases resting energy expenditure in humans with overweight or obesity in the presence or absence of concomitant GLP-1 infusion.[9] Similarly, glucagon can promote adipose tissue lipolysis under metabolic conditions where insulin signalling is reduced or suppressed.[10] Peripheral glucagon administration decreases meal size in rodents and humans;[7,11] however, administration of glucagon together with other anorectic peptides such as cholecystokinin (CCK) does not always produce additive or synergistic effects.[11] The ability of glucagon to induce weight loss and increase energy expenditure requires fibroblast growth factor 21 (Fgf-21) in mice, and short-term administration of glucagon increases FGF-21 levels in humans.[12] Indeed, FGF-21 mimics many of the metabolic actions of glucagon, and administration of FGF-21 produces improvements in insulin sensitivity, glucose homeostasis and dyslipidaemia, accompanied by increased energy expenditure and weight loss in preclinical models of diabetes mellitus and obesity.[13] Taken together, the majority of studies suggest that enhancing glucagon action in the brain is a useful strategy for the treatment of obesity.

GLP-1

GLP-1R expression has been detected throughout the body, including in pancreatic islet cells, lung, kidney, enteric neurons and the peripheral and central nervous system (CNS).[14] GLP-1R activation produces multiple hormonal and neural responses; for example, it stimulates insulin and inhibits glucagon secretion from islet cells in a glucose-dependent manner. GLP-1 also inhibits gastric emptying and gut motility through vagal afferents and conveys an anorectic signal to the brain that ultimately reduces food intake.[15] Furthermore, GLP-1 induces nausea via activation of aversive pathways in the brain.[16]

Pharmacological administration of GLP-1R agonists rapidly induces satiety, and sustained activation of GLP-1R, either via continuous administration of GLP-1 or via long-acting, high-molecular-weight GLP-1R agonists, results in weight loss in preclinical studies,[17] in patients with T2DM,[18] and in nondiabetic individuals with obesity.[19] Long-term GLP-1R activation results in tachyphylaxis of the inhibition of gastric emptying, which is potentially mediated in part through reduced inhibition

of the vagal tone.[20] Proposed therapeutic mechanisms for the induction of satiety include direct binding to hypothalamic GLP-1Rs, which reduces food intake.[21] High-molecular-weight GLP-1R agonists that do not readily cross the blood–brain barrier also rapidly activate c-Fos expression in multiple brain regions via ascending neural pathways in association with reduced food intake.[17,22]

Three degradation-resistant GLP-1R agonists approved for the treatment of T2DM—liraglutide, exenatide and lixisenatide—produce modest weight loss of 1–3 kg in clinical trials.[4] Although liraglutide is administered at daily doses of 1.2 mg or 1.8 mg for the treatment of T2DM, this agent is being studied at a once daily dose of 3 mg for the treatment of obesity. Patients treated with 3 mg of liraglutide once daily experienced a mean weight loss of 7.2 kg after 20 weeks of therapy.[19] About 25% of individuals with T2DM treated with GLP-1R agonists experience considerably greater weight loss than the usual 1–3 kg reported in clinical trials;[4] however, the genetic or molecular basis for this increased responsiveness remains poorly understood.

Oxyntomodulin

Oxyntomodulin transduces its glucoregulatory and anorectic actions through both the GLP-1R and GCGR.[23] However, although oxyntomodulin acts as a pharmacological agonist at both receptors, its binding affinity is reduced compared with that of GLP-1 and glucagon for their respective receptor.[24] Relative to pure GLP-1R agonists, oxyntomodulin activates the GLP-1R *in vitro* with ~100-fold decreased agonist potency.[23]

Oxyntomodulin induces weight loss in rodents and in patients with obesity by suppressing food intake and increasing energy expenditure.[25,26] The anorectic effects of acute central administration of native oxyntomodulin are blocked by the GLP-1R antagonist exendin(9–39) in rats,[25] preserved in *Gcgr*$^{-/-}$ mice, but abolished in *Glp1r*$^{-/-}$ mice.[23] These findings illustrate that oxyntomodulin requires GLP-1R signalling to reduce food intake. Although central administration of glucagon, GLP-1 or oxyntomodulin enhanced BAT thermogenesis and increased the expression of BAT genes important for energy expenditure in mice, these actions of oxyntomodulin were abolished in BAT of *Glp1r*$^{-/-}$ mice,[27] consistent with the importance of the GLP-1R for oxyntomodulin-regulated thermogenesis. Nevertheless, oxyntomodulin administration for 14 days attenuated weight gain in *Glp1r*$^{-/-}$ mice, and a GCGR antagonist reduced the extent of weight loss produced by oxyntomodulin administration in transgenic mice expressing a human GCGR.[28]

Oxyntomodulin administered three times daily reduced body weight by 2.4% over a 4-week period in nondiabetic patients with overweight or obesity.[29] Moreover, administration of oxyntomodulin to healthy individuals with overweight or obesity (BMI 25.1–39.0 kg/m^2) over 4 days was associated with an increase in nonresting energy expenditure.[30] Hence, oxyntomodulin reduces body weight through multiple receptors and mechanisms in both rodents and humans.

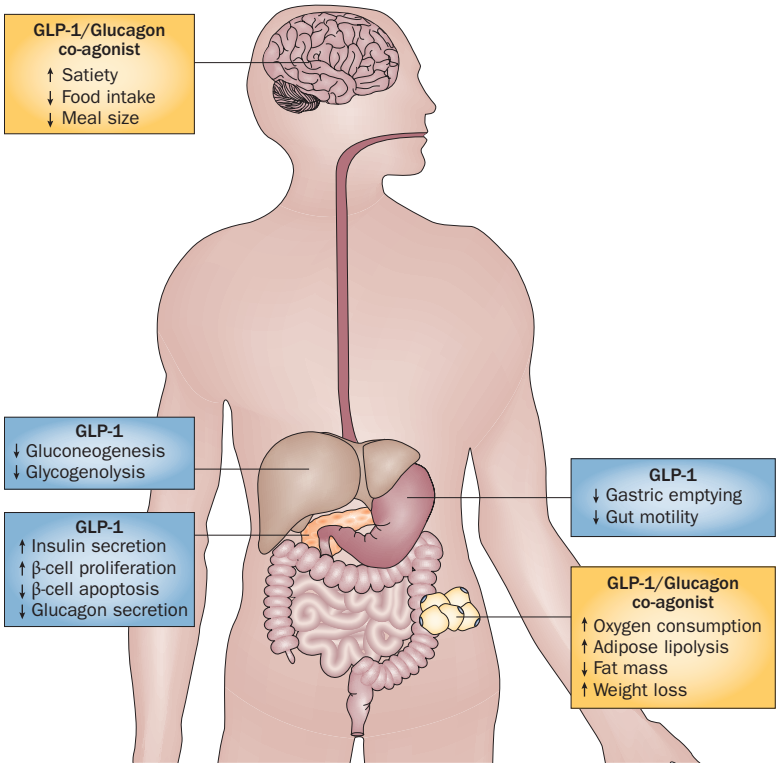

Figure 1 | Metabolic actions of GLP-1 (blue) and GLP-1/glucagon co-agonism (yellow) on mechanisms and tissues important for control of glucose homeostasis and body weight.

Co-agonism at the GCGR and GLP-1R

GCGR and GLP-1R are structurally related members of the class B receptor superfamily, with opposing mechanisms of action on the control of peripheral glucose homeostasis.[24] Nevertheless, as central activation of GCGR and GLP-1R both induce satiety, co-agonists that exhibit optimal ratios of GLP-1:glucagon agonism might produce additive or synergistic effects on weight loss whilst avoiding the hyperglycaemic effects of pure glucagon agonism (Figure 1). Co-administration of native glucagon and GLP-1 at subtherapeutic doses acutely potentiates neuronal c-Fos activation and reduction of food intake relative to the effects of either peptide alone.[31]

A series of glucagon-based peptides with amino acid substitutions at position 16, which increase activation of the GLP-1 receptor, have been conjugated to a 40-kDa polyethylene glycol (PEG) polymer to prolong their mechanism of action.[32] One of these glucagon–GLP-1 chimeric peptides, Aib2 C24 lactam 40k (named after an aminoisobutyric acid inserted to provide resistance to cleavage by dipeptidylpeptidase-4 [DPP-4]; a single side-chain lactam bridge to stabilize the secondary structure and enhance glucagon agonism; and the PEGylation at cysteine residue 24) is a nearly balanced co-agonist. In other words, this peptide exhibits almost equal potency at both the GLP-1R and GCGR relative to the native ligands GLP-1 and glucagon.[26] Conversely, the peptide Aib2 C24 40k is a long-acting molecule that is sevenfold more selective for GLP-1R than its more balanced, lactam-based version. At 1 week, a single injection

of the more balanced co-agonist Aib2 C24 lactam 40k in diet-induced obese mice decreased body weight by a remarkable 26%.[32] Administration of the unbalanced peptide Aib2 C24 40k was considerably less effective, with a decrease in body weight of 9%. Changes in body weight were associated with decreased fat mass and reduced food intake. Blood glucose levels were also significantly decreased with administration of both peptides, but more so with the balanced peptide.

Following four weekly injections of Aib2 C24 lactam 40k and Aib2 C24 40k, body weight of diet-induced obese mice was decreased by 28% and 20%, respectively; although reductions in food intake were less prominent, energy expenditure was increased after chronic treatment with either peptide.[32] The lactam-based co-agonist continued to reduce body weight in *Glp1r*$^{-/-}$ mice; however, glucose tolerance was impaired in these mice, which indicates that loss of the GLP-1R attenuates the glucoregulatory actions of GLP-1-biased co-agonists.[32] Similar relative efficacy on glycaemia and body weight was obtained following administration of the co-agonists in rats. These findings provide important proof of concept for the utility of co-agonism at the GLP-1 and GCGR as a treatment of obesity (via reduced food intake and increased energy expenditure).

The metabolic activity of a protease-resistant dual GLP-1R–GCGR long-acting agonist, DualAG, was compared with that of a GLP-1R agonist, GLPAG, in mice with diet-induced obesity.[33] The pharmacokinetic profiles of both peptides were extended through linking of cholesterol side chains at the C-terminus. The DualAG peptide produced superior weight loss and greater reductions in food intake than the GLPAG peptide. Acute peptide administration decreased plasma triglycerides, increased ketone bodies and expression of hepatic genes associated with gluconeogenesis and fatty acid oxidation, including *FGF21*.[33] The metabolic actions of DualAG were partially attenuated in both *Gcgr*$^{-/-}$ and *Glp1r*$^{-/-}$ mice, consistent with the importance of both receptors for transduction of the benefits of the co-agonist.

The relative importance of the GCGR versus the GLP-1R for the metabolic actions of oxyntomodulin was examined by creating a mutation at position 3 of oxyntomodulin, which converts a glutamine (Q) into a glutamic acid (E).[28] The peptide analogue OXMQ3E activates the GLP-1R with similar potency as oxyntomodulin, but with no agonist or antagonist activity at the GCGR. Oxyntomodulin administration produced greater weight loss than did OXMQ3E administration (14% versus ~11% reduction in body weight over 14 days) in mice with diet-induced obesity. Unlike oxyntomodulin, OXMQ3E did not produce weight loss in *Glp1r*$^{-/-}$ mice. Oxyntomodulin, but not OXMQ3E, stimulated liver ketogenesis in wild-type and *Glp1r*$^{-/-}$ mice but not in *Gcgr*$^{-/-}$ mice.[34] Furthermore, oxyntomodulin produced superior weight loss and lowering of lipid levels compared with OXMQ3E. The ability of oxyntomodulin to reduce body weight was attenuated in mice fed compound A, a GCGR antagonist. Importantly, OXMQ3E improved glucose tolerance at lower doses than oxyntomodulin.[28] To deduce the relative importance of the GCGR versus the GLP-1R for oxyntomodulin action, oxyntomodulin was administered to wild-type and *Glp1r*$^{-/-}$ mice. Oxyntomodulin administration impaired control of glycaemia despite enhancing insulin secretion through the GCGR during a hyperglycaemic clamp in *Glp1r*$^{-/-}$ mice.[34] Hence, unopposed GCGR signalling in the liver in the absence of GLP-1R activation compromises the glucoregulatory potential of oxyntomodulin-based co-agonists.[34]

The metabolic effects of transient administration of native glucagon and GLP-1, infused alone or together for 45 min, were assessed in healthy human volunteers with overweight or obesity. Peak levels of glucagon and GLP-1 achieved during the infusion were ~906 ng/l and 340 ng/l, respectively.[9] Both glucagon and GLP-1 reduced levels of nonesterified free fatty acids and increased insulin levels. Plasma glucose levels rose with glucagon infusion and the glucagon-stimulated increase in plasma glucose level was attenuated by co-administration of GLP-1. Resting energy expenditure increased modestly with glucagon alone, with no detectable change in core temperature, and co-administration of GLP-1 had no further effect on energy expenditure but did significantly reduce plasma levels of total and acyl ghrelin. Information on whether the acute metabolic effects of a glucagon–GLP-1 co-agonist would be sustained with chronic administration in humans is currently lacking.

Gastric inhibitory polypeptide

The best characterized actions of gastric inhibitory polypeptide (GIP) include stimulation of glucose-dependent insulin secretion and insulin biosynthesis, and expansion of β-cell mass via stimulation of β-cell growth and preservation of β-cell survival.[2,35]

Unlike GLP-1, the insulinotropic action of GIP is substantially impaired or absent in the majority of patients with T2DM. The expression of the *GIPR* gene, which encodes the GIP receptor, is downregulated under conditions of hyperglycaemia, and GIPR signalling seems to be impaired in the diabetic β cell. Nevertheless, the resistance of the diabetic β cell to GIP is reversible, as a 4-week treatment with insulin improved glucose control, lowered levels of HbA_{1c} to ~7% and restored β-cell GIP responsiveness in patients with T2DM.[36] GIP also promotes adipose tissue accretion and increases plasma levels of adipokines such as resistin, which impairs insulin action.[37] GIP stimulates glucagon secretion under hyperglycaemic conditions in patients with T2DM;[38] whether these stimulatory actions on the α cell are sustained with chronic administration of GIPR agonists is not known. Attenuation of GIP action or genetic disruption of *GIPR* in preclinical studies is associated with paradoxical improvements in glucose control, resistance to weight gain and enhanced insulin sensitivity.[2,39] Hence, the extent to which activation or reduction of GIP action represents a preferred strategy for the treatment of obesity and/or diabetes mellitus in humans remains uncertain.

Degradation-resistant N-terminally modified GIPR agonists exhibit robust glucoregulatory actions in

preclinical studies. The N-terminally protected analogue of GIP N-acetyl-GIP(LysPAL16) administered once daily (12.5 nmol/kg body weight) for 2 weeks decreased plasma glucose and HbA_{1c} levels and improved glucose tolerance in leptin-deficient *ob/ob* mice, with no changes in body weight. By contrast, the degradation-resistant GIPR agonist (D-Ala^2)GIP increased weight and plasma resistin levels and impaired insulin action in mice fed a high-fat diet.[40] Hence, variability in metabolic benefits attributable to activation of the GIPR reflects the potency of the GIPR agonist and the specific experimental model.

Given the overlapping metabolic benefits that arise from either activation or reduction of GIPR signalling in patients with obesity or T2DM, combination therapy studies employing GIP-related peptides have used both agonist and antagonist strategies. Combined treatment of mice fed a high-fat diet with N-acetyl-GIP and the GLP-1R agonist exendin-4 for 12 days, after an initial 12-day regimen of exendin-4 alone, resulted in a modest increment in weight loss with no additional benefit in glucose control compared with treatment with exendin-4 alone.[41] By contrast, the combination of liraglutide with 100 nmol/kg of N-acetyl-GIP(Lys37Myr)—N-acetyl-GIP with myristic acid conjugated at Lys37—for 21 days was more potent at lowering plasma glucose but did not exhibit any additive benefit on reduction of body weight compared with the administration of either peptide alone in *ob/ob* mice.[42] Similarly, treatment of diet-induced obese mice with the CCK analogue (pGlu-Gln)-CCK and the PEGylated GIPR antagonist (Pro^3)GIP for 34 days did not produce any additive benefit on control of body weight or glucose homeostasis relative to treatment with either agent alone.[43] Furthermore, combination therapy employing the GLP-1R agonist (D-Ala^8)GLP-1 together with (Pro^3)GIP had no additive benefit on glycaemic control or body weight relative to the single peptides alone over a 48-day treatment period in mice fed a high-fat diet.[44] Combined administration of (Pro^3)GIP and the cannabinoid receptor 1 antagonist AM251 produced no added benefit relative to metabolic effects obtained with either single agent alone over a 22-day treatment period in diet-induced obese mice.[45] Nevertheless, improved metabolic outcomes in preclinical studies with GIP–GLP-1 or GIP–GLP-1–glucagon co-agonists have been reported; however, full experimental details have not yet been forthcoming.[46]

No data exist on the metabolic consequences of sustained activation of GIPR signalling in humans. Nevertheless, short-term combination therapy of native GLP-1 and GIP infused into patients with T2DM (mean age 60 years, BMI 30 kg/m^2) for 6 h did not show additive benefit for the stimulation of insulin secretion compared with the effects of GLP-1 alone. In fact, co-infusion of GIP and GLP-1 antagonized the suppression of plasma glucagon levels achieved with GLP-1 alone.[47]

Gastrin

Classic gastrin action includes promotion of acid secretion from parietal cells of the stomach. The gastrin peptide and the CCK receptor type B (CCK-BR) are both transiently expressed in the fetal pancreas at a time of increased β-cell proliferation, and transgenic mice with overexpression of gastrin and transforming growth factor α in the pancreas exhibit increased islet and β-cell mass.[48] Gastrin monotherapy (2.5 nmol/kg per hour) infused continuously over 3 days in duct-ligated rats produced expansion of β-cell mass without detectable changes in proliferation, apoptosis or β-cell size, consistent with an effect of gastrin on neogenesis or β-cell differentiation.[49]

Neither gastrin nor GLP-1 alone restored glycaemia to normal levels in nonobese diabetic (NOD) mice; however, a combination of GLP-1 (10 μg/kg or 100 μg/kg) and gastrin (1.5 μg/kg) for 3 weeks, markedly improved glycaemia in NOD mice, and the majority of NOD mice receiving combination therapy remained normoglycaemic for weeks after cessation of the gastrin–GLP-1 therapy.[50] The improved glucose control was associated with increased pancreatic insulin content and β-cell mass to 74% and 53% of normal, respectively. These findings were attributed to immunomodulatory actions of the gastrin–GLP-1 combination, as transferred splenocytes from NOD mice rendered normoglycaemic by gastrin–GLP-1 therapy protected immunodeficient NOD-SCID (severe combined immunodeficiency) mice from diabetes mellitus.[50]

Improved glucose control and expansion and/or preservation of functional β-cell mass has been achieved using gastrin–GLP-1 to treat NOD-SCID mice with transplanted human islets and by combining gastrin with DPP-4 inhibitors in animal models of diabetes mellitus. The combination of induced mixed chimaerism using anti-CD3/CD8 and administration of gastrin and epidermal growth factor augmented β-cell neogenesis and replication and reversed established type 1 diabetes mellitus (T1DM) in old NOD mice.[51] The pharmacological actions of the gastrin–GLP-1 dual agonist ZP3022 were examined in leptin-receptor-deficient *db/db* mice, a model of insulin resistance and T2DM. ZP3022 acts as an agonist at both the GLP-1R and CCK-BR; in *db/db* mice, this dual agonist produced a more sustained glucoregulatory effect than liraglutide, even after cessation of therapy.[52] The salutary effects of ZP3022 were associated with a significant increase in pancreatic insulin content and β-cell mass, to a greater extent than that seen with liraglutide alone. Hence, gastrin–GLP-1 combination therapy produces considerable glucoregulatory activity in experimental models of both T1DM and T2DM.

Several studies have assessed the effects of proton-pump inhibitors, which block gastric acid production and raise levels of endogenous gastrin, on glycaemic control in animals and in humans with diabetes mellitus. Addition of pantoprazole to the treatment regimen of patients with T2DM receiving metformin with or without a sulfonylurea significantly increased levels of gastrin, improved β-cell function (as determined by homeostatic model assessment) by 30%, and reduced levels of HbA_{1c} over a 12-week treatment period.[53] By contrast, daily administration of 40 mg of esomeprazole to patients with T2DM managed by diet or oral antidiabetic agents alone had no effect on HbA_{1c} or insulin levels over a 12-week treatment period.[54] No published

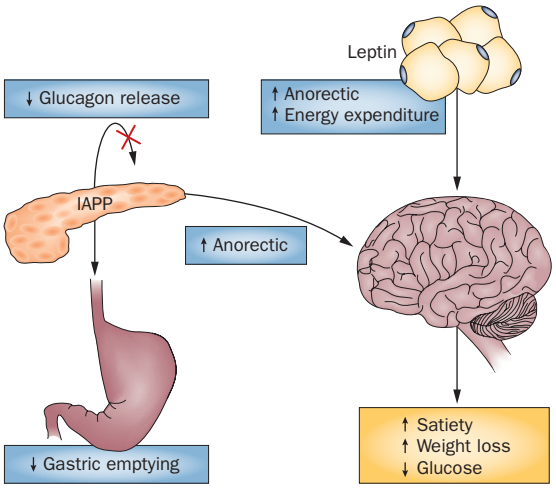

Figure 2 | Mechanisms for leptin and IAPP co-agonism in the control of body weight and glucose homeostasis. Both proteins act in the brain to induce satiety and peripherally on metabolic and glucoregulatory pathways.

studies have thus far demonstrated the glucoregulatory efficacy of GLP-1–gastrin agonism in humans with diabetes mellitus; hence, whether combined activation of the GLP-1R and CCK-BR represents a viable strategy for co-agonism requires additional investigation.

Islet amyloid polypeptide and leptin

Administration of exogenous islet amyloid polypeptide (IAPP), which is also known as amylin, decreases postprandial blood glucose levels through inhibition of gastric emptying and suppression of glucagon secretion.[55] IAPP also induces satiety, which leads to weight loss after repeated administration in obese individuals with or without diabetes mellitus. Activation of IAPP receptors in the area postrema leads to engagement of neural circuits in the nucleus of the solitary tract, parabrachial nucleus and the amygdala, resulting in satiety and decreased food intake.[55]

Pramlintide is a stable equipotent IAPP analogue with proline amino acid substitutions at positions 25, 28 and 29, which results in a bioactive peptide with enhanced solubility that exhibits markedly reduced potential, relative to native IAPP, for amyloid fibril formation. Pramlintide is administered subcutaneously and has been approved for the treatment of patients with T1DM or T2DM who have not achieved optimal glycaemic control despite being on insulin therapy. Pramlintide decreases food intake and body weight in both diabetic and nondiabetic individuals with obesity.[56] The observation that pramlintide combined with leptin produces synergistic effects on weight loss in preclinical studies has sparked considerable interest in the biology of leptin and IAPP co-agonism for the treatment of obesity (Figure 2).

Initial enthusiasm for the use of leptin as a treatment of obesity was tempered by subsequent observations that circulating levels of leptin were significantly elevated in obese animals and humans. Evaluation of responses to leptin therapy revealed resistance to leptin action in experimental and clinical states of obesity.[57] The biology of leptin action in preclinical studies greatly depends on the experimental model under analysis. Indeed, much enthusiasm currently exists for assessing leptin as a therapy for T1DM; however, data on the use of leptin alone in individuals with T2DM have been less compelling. This subject has been reviewed in detail elsewhere.[58]

Multiple approaches have attempted to restore leptin sensitivity; however, preclinical evidence and human investigation support the possibility of leptin and IAPP co-agonism for the treatment of obesity. Acute central administration of leptin and peripheral administration of IAPP synergistically inhibit food intake in rats.[59] Subsequent studies demonstrated that IAPP restored leptin responsiveness in obese rats, and co-administration of peripheral leptin and IAPP produced additive and/or synergistic effects on food intake and weight loss in rats with diet-induced obesity.[60] The selectivity of the IAPP–leptin interaction was highlighted by complementary experiments that failed to demonstrate similar co-agonism efficacy when combining either a GLP-1R agonist or PYY(3-36) with leptin, with comparable negative findings confirmed by other reports.[61] Nevertheless, subsequent studies demonstrated restoration of leptin responsiveness and enhanced weight loss in obese rats using either the GLP-1R agonist exendin-4, or FGF21, in combination with leptin administration.[62] Hence, a better understanding of optimal dosing regimens required to achieve synergistic efficacy with specific co-agonist regimens seems desirable.

Importantly, synergistic effects of pramlintide and metreleptin were observed in a 20-week-long clinical trial in patients with overweight or obesity (BMI 27–35 kg/m^2).[60] Although reduction of food intake might represent the dominant mechanism underlying the effects of IAPP–leptin co-agonism, preclinical studies reveal a disproportionately greater reduction in adipose tissue with IAPP–leptin therapy than that observed in pair-fed control animals.[63]

In a second proof-of-concept clinical study, the effects of IAPP–leptin co-agonism were examined in 177 nondiabetic individuals with obesity or overweight (mean BMI 32 ± 2.1 kg/m^2, mean age 39 years, 63% women).[64] Patients were pretreated with pramlintide for 4 weeks (180 μg twice daily for 2 weeks and 360 μg twice daily thereafter) together with a calorie reduction of 40%. The study participants were then randomly allocated to one of three groups for an additional 20 weeks: pramlintide (360 μg twice daily); metreleptin (5 mg twice daily); or pramlintide plus metreleptin (360 μg plus 5 mg twice daily, respectively). Weight loss was significantly greater in the combined treatment group (12.7%) than in the pramlintide (–8.4%) or metreleptin (–8.2%) groups.[64] The most common adverse events reported in the combined treatment group were reactions at the injection site (58.9%) and nausea (12.5%); however, in most patients, nausea subsided after 5 weeks of treatment.[64] Further evaluation of the potential clinical efficacy of pramlintide–leptin co-agonism awaits resolution of potential safety issues surrounding detection of anti-leptin antibodies in a small number of trial participants.[65]

PYY

PYY circulates in two active forms, PYY(1-36) and PYY(3-36). Cleavage of PYY(1-36) by DPP-4 produces PYY(3-36). These two peptides exert differential bioactivity through activation of different NPY receptor subtypes. PYY(1-36) acts through the Y_1 and Y_5 receptors in the CNS and might stimulate appetite, whereas PYY(3-36) activates Y_2 receptors to inhibit appetite and promote weight loss.[66] PYY infusions in humans delay gastric emptying and colonic transit time;[67] hence, PYY arising from the distal gut contributes to the 'ileal brake' mechanism.

Short-term administration of PYY(3-36) inhibits food intake over 24 h in individuals with obesity;[68] however, little evidence exists to suggest a considerable reduction in body weight with sustained administration of PYY agonists in individuals with obesity. In a randomized study, 133 patients aged 18–45 years with obesity (BMI 30–43 kg/m^2) were treated with 200 μg or 600 μg of PYY(3-36) or with placebo. PYY(3-36) was administered as a 0.1 ml intranasal spray 20 min before breakfast, lunch and dinner for 12 weeks, following 2 weeks of a hypocaloric diet (25% caloric deficit) and exercise regimen that was continued throughout the study. The least-squares mean difference (95% CI) in body weight between individuals treated with the 200 μg dose or placebo was −1.2 kg, which was not significant. Clinical adverse events led to discontinuation in 27 of the 42 patients in the 600 μg group; the main reason for discontinuation was nausea.[69]

Combination of IAPP with PYY(3–36) has demonstrated more durable weight loss in rodent models compared with human studies.[70] Mice with diet-induced obesity were treated with vehicle, 300 μg/kg daily of IAPP, 1,000 μg/kg daily of PYY(3–36), or both for 14 days. Cumulative food intake and body weight were significantly reduced in all three groups; however, the IAPP–PYY combination produced significantly more weight reduction than either agent alone. Similar results were obtained in rats with diet-induced obesity, which maintained their metabolic rate despite greater weight loss with IAPP–PYY co-administration than with a single agent.[70]

Safety considerations

Evaluation of co-agonist efficacy in preclinical studies is focused on changes in food intake, glycaemia and body weight; much less attention is paid to the effects of these new molecules on behaviour, bone formation and resorption or reproductive biology. Indeed, the considerable enthusiasm for FGF21-based therapies for the treatment of diabetes mellitus and obesity must be balanced by preclinical observations that link FGF-21 activity to attenuation of chondrocyte activity, potentiation of PPAR-γ-like activity on osteoblasts and reduced bone mass.[71] Furthermore, the majority of regulatory peptides and adipokines such as leptin are active in the CNS, where they can modify neuronal circuits controlling reward systems and behaviour. As manipulation of pathways to reduce food intake might be associated with enhanced anxiety or depression, careful characterization of the behavioural effects of co-agonists is warranted.

The receptors for glucagon, GLP-1, GIP, leptin and IAPP are expressed and functional in the heart and blood vessels. Whether specific co-agonists affect blood pressure, heart rate, vascular tone and the function of the normal and ischaemic heart has only been addressed in limited, short-term, *ex vivo* studies to date.[72] Short-term administration of the glucagon–GLP-1 co-agonist ZP2495 increased glucose oxidation, glycolytic rates and cardiac performance *ex vivo* in the insulin-resistant rat heart;[72] however, limited data exist on the cardiac effects of sustained co-agonist administration in preclinical models of atherosclerosis, diabetes mellitus or ischaemic heart disease. As GLP-1 increases heart rate in patients with diabetes mellitus and/or obesity,[73] the potential for additive chronotropic effects with one or more co-agonist combinations requires careful assessment.

The use of GLP-1R agonists has been associated with case reports of pancreatitis, although no mechanism linking GLP-1R activation to pancreatic inflammation has been reported.[74] Analyses of health-care databases for a putative association between incretin-based therapies and pancreatitis have not yielded evidence linking GLP-1R agonists with increased rates of pancreatic injury.[74] GLP-1R activation also increases cell proliferation, leading to pancreatic ductal proliferation, expansion of the gut epithelial mucosa, and C-cell hyperplasia and medullary thyroid carcinoma in preclinical studies.[74] Hence, the extent to which combination of a GLP-1 epitope with a second metabolic hormone in a co-agonist will modify the augmentation of cell proliferation requires further study.

As is the case with all protein therapies, the potential for immunogenicity, arising in response to a novel co-agonist with unique molecular structure, and antigenicity must be considered. Similarly, the potential for loss of efficacy over time, arising from receptor desensitization and tachyphylaxis, might require careful consideration of optimal dosing protocols for sustained and maximal efficacy.

The impressive weight loss reported with many co-agonists in preclinical studies is often associated with engagement of pathways converging on BAT, activation of the sympathetic nervous system and increased energy expenditure. Whether these mechanisms will be completely preserved and safely modified in obese, insulin-resistant humans with diabetes mellitus is not at all evident. The long-term safety of co-agonists that increase energy expenditure in humans requires careful investigation in large groups of individuals with obesity and risk factors for cardiovascular disease. Our understanding of factors and molecules that control white and brown adipocyte number, mass and function in humans is incomplete and changing rapidly. Nevertheless, despite these caveats and limitations, the majority of patients with obesity and diabetes mellitus remain suboptimally treated by current therapeutic approaches. The highly promising data emerging from the evaluation of newly developed co-agonists strongly supports ongoing efforts towards the design and evaluation of novel co-agonist therapies for the treatment of metabolic disease.

Conclusions

As dozens of peptides and proteins reduce food intake, decrease fat mass and/or exert favourable metabolic effects on control of glycaemia and dyslipidaemia, considerable experimentation is ongoing to characterize the potential metabolic benefits of different co-agonist combinations. Furthermore, the use of 'triple therapy', ideally combining three distinct biologically active epitopes in a single molecule, is also being explored.[46] Additional co-agonist approaches under study include: leptin plus FGF21 or exendin-4;[62] FGF21 plus GLP-1R agonists; GLP-1R agonists plus cannabinoid receptor 1 antagonists; and IAPP in combination with naltrexone–bupropion therapy.[75] And the list of co-agonist candidates continues to expand.

Presently, the molecular mechanisms underlying the enhanced efficacy of co-agonists remain poorly understood. Whether key receptors of interest targeted by co-agonists are expressed on the same cell type and directly engage complementary signalling pathways within a single cell or group of neurons remains unclear. Alternatively, co-agonists might simultaneously activate distinct, yet similar, pathways in different cells and brain regions, resulting in enhanced efficacy following convergence of these independently generated signals. Finally, whether co-agonists primarily exert their central effects via direct penetration of the CNS or whether they initiate signals peripherally that are subsequently transmitted via neural pathways to the CNS requires more careful assessment.

Review criteria

A search for original articles published between 2000 and January 2013 was performed in PubMed. The search terms used were "co-agonist", "oxyntomodulin", "GLP-1", "GIP", "amylin", "glucagon", "leptin", "peptide hormone", together with the terms "obesity", "diabetes" and "weight loss". All identified articles were English-language, full-text publications.

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Author contributions

Both authors contributed equally to all aspects of the article.