Action and therapeutic potential of oxyntomodulin*

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ABSTRACT

Oxyntomodulin (OXM) is a peptide hormone released from the gut in post-prandial state that activates both the glucagon-like peptide-1 receptor (GLP1R) and the glucagon receptor (GCGR) resulting in superior body weight lowering to selective GLP1R agonists. OXM reduces food intake and increases energy expenditure in humans. While activation of the GCGR increases glucose production posing a hyperglycemic risk, the simultaneous activation of the GLP1R counteracts this effect. Acute OXM infusion improves glucose tolerance in T2DM patients making dual agonists of the GCGR and GLP1R new promising treatments for diabetes and obesity with the potential for weight loss and glucose lowering superior to that of GLP1R agonists.

Keywords Oxyntomodulin; Glucagon; GLP-1; Glucose metabolism; Body weight

1. INTRODUCTION

T2DM therapies such as metformin, sulfonylureas (SU), glinides and insulin result in progressive deterioration of glycemic control associated with β-cell decline [1] and weight gain [2]. Incretin-based therapies such as glucagon-like peptide-1 receptor (GLP1R) agonists and dipeptidyl peptidase-4 (DPP-4) inhibitors lower blood glucose primarily by promoting glucose dependent insulin secretion and by inhibiting glucagon secretion [3]. The glucagonostatic effect of GLP1R activation is likely mediated by a local action of somatostatin released by δ cells that inhibits glucagon secretion by pancreatic α cells [4,5]. The delay of gastric emptying by GLP-1 has been involved in the postprandial regulation of glucose absorption [6,7]. However, Nauck et al. [8] demonstrated in healthy volunteers that GLP-1-induced delay in gastric emptying is subject to rapid tachyphylaxis. As a consequence, postprandial glucose control by GLP-1 is attenuated during its chronic administration [8]. Thus far, these therapies are providing durable glycemic control with low risk of hypoglycemia, improved insulin resistance, pancreatic function and modest body weight-loss at tolerated doses [9]. GLP1R agonism has been shown to lower plasma glucose in advanced type 2 diabetes long after sulfonylurea secondary failure [10]. GLP1R activation may delay or prevent secondary SU failure [11]. With such promising results, the next generation of diabetes drugs will likely focus on combined activation of more than one receptor ideally resulting in beneficial cardio-renal effects.

2. OXYNTOMODULIN

Oxyntomodulin (OXM) is a 37-amino acid peptide hormone secreted from the gut together with GLP-1 following nutrient ingestion [12–18]. OXM is mainly produced in gut endocrine L-cells by processing of the preproglucagon precursor by prohormone convertase 1/3 [12–15,19,20] (Figure 1). While the existence of gut ‘glucagon-like substances’ was reported in gut extracts since 1948 [21], it was discovered in 1981 that one moiety with ‘glucagon-like immunoreactivity’ [22] was formed by the sequence of glucagon and a C-terminal octapeptide extension (IP-1, intervening peptide, Figure 1). This moiety was named oxyntomodulin for its ability to modulate gastric acid secretion in gastric oxyntic glands [23–26].

3. RECEPTORS AND SIGNALING IN VITRO

OXM is reported to be a full agonist in cell lines over expressing the human GLP1R and GCGR-mediated cAMP accumulation although with reduced affinity compared to GLP-1 and glucagon [27–32]. It was found to be a partial agonist in recruiting β-arrestin and G-protein-coupled receptor kinase 2 to the GLP1R. OXM has been proposed as a GLP1R-biased agonist relative to GLP-1 as it has less preference towards cAMP signaling relative to phosphorylation of ERK1/2, but similar preference for calcium relative to Ca2+ [31]. If these findings translate in vivo, the GLP1R-mediated effects of OXM could differ from that of GLP-1 [32]. Administration of OXM increased c-fos in the hypothalamus, but not in the brainstem [33], and OXM and GLP-1 result in differential neuronal activation in the hypothalamus [34,35]. Consistent with these data, peripheral administration of glucagon or GLP-1 or co-administration of glucagon and GLP-1 activates similar appetite regulating centers in the brainstem and amygdala [36]. It is unclear if these differences are due to engagement of additional receptors and/or the reported difference on cell signaling.

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GLP1R signaling by OXM or simply a different brain penetration and affinity at the receptors.

4. EFFECTS OF OXYNTOMODULIN ON BODY WEIGHT AND GLUCOSE METABOLISM

OXM causes weight loss in humans [37,38] and rodents [30,39,40]. Overweight and obese subjects receiving subcutaneous administration three times daily of OXM (400 nmol pre-prandially) over a 4-week period resulted in an average weight loss of 2.3 kg [38]. The endogenous levels of ‘OXM-like immunoreactivity’ (OLI) (see review in [41]) increased ~10-fold (972 ± 165 pmol/l) 30 min after the self-injection. These levels are comparable to those detected in patients following gastric bypass surgery [42] and in several conditions and procedures associated with anorexia such as tropical malabsorption [43] and small intestinal resection [44]. Moreover, in preclinical species, chronic treatment with OXM results in superior weight loss and comparable glucose lowering to a GLP1R-selective peptide [45–47]. OXM improves glucose metabolism during a glucose tolerance test in mice [48,49]. The acute improvement of glucose metabolism by OXM was recently confirmed in humans in a randomized, double-blind, placebo-controlled, crossover trial, evaluating the effect of OXM in a graded glucose infusion (GGI) performed in T2DM volunteers [50]. OXM was infused (3 pmol/kg/min) to match the plasma concentrations achieved in post-prandial state in the chronic weight loss study performed on overweight and obese volunteers [38]. At matched glucose infusion rates, OXM significantly increased insulin secretion and glucose lowering versus placebo [50].

5. MECHANISMS INVOLVED IN THE EFFECTS OF OXM ON ENERGY AND GLUCOSE METABOLISM

OXM was shown to lower food intake and reduce body weight as well as increase core temperature compared to a pair-fed group suggesting that OXM increases energy expenditure [39,40]. This effect was later confirmed by indirect calorimetry [30]. The acute anorectic effect of OXM was then demonstrated in humans where continuous infusion reduced ad libitum energy intake in the absence of nausea or effects on food palatability [37]. Later Wynne et al. [38] showed that the reduction in food intake was seen and retained during chronic administration in overweight and obese healthy volunteers (Figure 2). In a similar population, four-day subcutaneous self-administration of pre-prandial oxyntomodulin increased energy expenditure while reducing energy intake [51]. Therefore the body weight-lowering effects of OXM likely involve suppression of food intake and an increase in energy expenditure [30,38,51]. In addition to the established glucose-dependent insulin secretion action of OXM on the pancreas (Figure 2) [52], another mechanism that potentially plays a role on energy intake and glucose metabolism is gastric emptying. Intravenous infusion of OXM reduced gastric emptying in humans (Figure 2) [53]. Further studies are required to confirm these findings and to understand if reduction in gastric emptying is involved in the acute and long-term metabolic effects of OXM.

6. RECEPTORS INVOLVED IN THE EFFECT OF OXYNTOMODULIN

6.1. Body weight

OXM inhibits food intake and stimulates energy expenditure in rodents and in human subjects [37–40]. However, the receptors involved in the

![Figure 1: Preproglucagon. Preproglucagon is proteolytically cleaved in a tissue-specific manner. Post-translational processing in the gut and brain by prohormone convertases results in the secretion of GLP-1 and GLP-2, while the glucagon sequence remains in a larger peptide, glicentin or glicentin-related pancreatic peptide (GRPP) and oxyntomodulin. MPGF, major proglucagon fragment.](image)

![Figure 2: Effects of oxyntomodulin in humans.](image)
body-lowering action of OXM are not fully delineated. The anorectic effects of central administrations of OXM are abolished by co-administration of the GLP1R antagonist, exendin(9–39), and are not observed in Glp1r<sup>−/−</sup> mice, suggesting that the acute central effect of OXM on food intake is mediated by the GLP1R [30,54,55]. Other effects of OXM, including stimulation of heart rate and energy expenditure, appear to be independent of GLP1R in vivo [30,54]. As OXM agonizes GLP1R and GPCR in vitro [27–32] and because increases in heart rate and energy expenditure were reported following GPCR activation [56], the differential effect of OXM vs. GLP1 could be mediated by activation of the GPCR.

Recently, two publications [45,46] expanded the initial findings on the mechanism of action of OXM and demonstrated that OXM has glycogenolytic properties in perfused mice liver [46]. Using an equipotent GLP1R agonist peptide obtained with a mutation of glutamate (OXM<sub>Q3E</sub>) in position 3 that does not activate the GLP1R, researchers were able to demonstrate that OXM, not OXM<sub>Q3E</sub>, stimulated ketogenesis in wild-type mice and in Glp1r<sup>−/−</sup> mice, but not in Gcr<sup>−/−</sup> mice [45]. These data show that OXM functionally activates the GPCR ex vivo and in vivo [45,46]. Chronic treatment with OXM resulted in superior body weight lowering and comparable glucose-lowering to equimolar amounts of OXM<sub>Q3E</sub> [46]. Moreover, OXM significantly decreased body weight gain in lean Glp1r<sup>−/−</sup> mice and chronic blockade of the GPCR with a small molecule GPCR antagonist (Cpd A) during OXM infusion reduced the ability of OXM to decrease body weight [46]. These data, using genetic and pharmacological approaches, suggest that the body-weight lowering action of OXM involves GLP1R and GPCR activation [45,46]. Nevertheless these data are not conclusive as in the same study Cpd A alone in feed resulted in significant body-weight reduction thereby limiting the interpretation of each receptor’s contribution to the body weight-lowering effect of OXM [46].

6.2. Glucose metabolism

OXM acutely improves glucose metabolism in mice [48,49]. However, consistent with the known stimulatory effect of glucagon on hepatic glucose production, it was demonstrated that GLP1R-agonism exerts better glucose lowering than OXM at equimolar doses in a glucose challenge [45]. During a mouse hyperglycemic clamp, OXM infusion reduced the exogenous glucose required to maintain the hyperglycemic levels in Glp1r<sup>−/−</sup> vs. Gcr<sup>−/−</sup> and wild-type mice despite increased plasma insulin levels. These data demonstrated that activation of GPCR contributes to the insulinotrophic effect of OXM and partially attenuates its beneficial effects on glucose metabolism [45]. These findings are consistent with increased glucose production observed in mice treated with OXM during a hyperinsulinemic-euglycemic clamp [49]. A recent report [57] demonstrated in rats that intracerebroventricular glucagon administration improves whole-body glucose metabolism. If these data translate to higher species, it is possible that central GPCR signaling may contribute to the improvement of glucose metabolism in animals treated with OXM.

Although previous data have shown that the glucagon receptor is involved in the body weight and glucose lowering action of OXM in addition to GLP1R, OXM may activate additional receptors. Oxyntomodulin was shown to inhibit pancreatic secretion through the nervous system in rats [58]. While glucagon can depress pancreatic exocrine secretions in normal animals and humans, it is still unclear how glucagon exerts this effect [59]. As previously mentioned, the differential neuronal activation observed following OXM, GLP-1 and glucagon administration seems to support a dissociation between the effects of oxyntomodulin and those of GLP-1 and glucagon [33–36]. Intravenous administrations of OXM and glucagon have also been reported to increase intestinal glucose uptake in rats [60]. While in vivo data may be confounded by differential levels of circulating insulin, OXM was described as a more potent regulator than glucagon in stimulating intestinal glucose absorption in the isolated small intestine despite being 1–2 orders of magnitude less potent at the GPCR than glucagon [61]. Because GLP-1 does not stimulate glucose absorption and an increase in hexose transport has been previously described for glucagon-like peptide-2 (GLP-2) and glucose-dependent insulinoletic peptide (GIP) [62,63], OXM could engage additional G-protein-coupled receptors (GPCR) of the secretin like (class B) family such as GLP-2 and GIP receptors [64,65]. Treatment of BHK-GIPR or BHK-GLP2R rat cells with OXM had no effect on cAMP production, whereas BHK cells that express the rat GLP-1 or glucagon receptors exhibited significant increases in cAMP accumulation in response to treatment with OXM [30]. Taken together, these data suggest that OXM may engage additional receptors but that additional downstream pathways aside from cAMP may be involved [31]. OXM also delayed gastric emptying in humans [53] but not in mice [48] adding a layer of complexity in interpreting receptors activated by OXM. Further investigation is required on these data. It is possible that differences interspecies may further complicate the interpretation of the mechanisms involved in the effects of OXM.

7. POTENTIAL MEDIATORS OF THE METABOLIC EFFECTS OF OXM

While the anorectic effect of GLP-1 in humans seems to involve an intact vagus nerve [66], it is unclear how glucagon receptor activation suppresses food intake. The anorectic effect of glucagon is abolished following selective hepatic vagotomy in rats suggesting that glucagon acts in the liver to produce a satiety signal that is transmitted to the brain by the vagus nerve [67]. Recently, Tan and colleagues reported an inhibitory effect on plasma levels of the anorexigenic hormone ghrelin in humans during short-term infusion of GLP-1 and glucagon [68]. While part of this effect could be mediated by insulin stimulation, central administration of OXM suppresses circulating ghrelin-like immunoreactivity in rodents [69] and intravenous infusion of OXM at a dose that did not stimulate insulin secretion lowered ghrelin in humans [37]. Thus, it appears that while GLP-1 suppresses plasma ghrelin in humans via insulin secretion in the late postprandial period [70], OXM and GLP-1/ghcagon co-administration exert a ghrelin-lowering effect independent of insulin (Figure 2). Ghrelin stimulates appetite in humans [71,72], but it is unclear whether ghrelin suppression contributes to the anorectic effect of OXM in humans as no changes in plasma ghrelin were detected during a 28-day weight loss study performed in overweight and obese volunteers [38].

In the aforementioned study, during infusion of GLP-1 and glucagon in healthy overweight and obese volunteers [68] GLP-1 blunted the hyperglycemic effect of glucagon compared to the individuals receiving glucagon alone. An initial rise in glucose levels diminished over time [68]. The authors suggested that the hyperglycemic effect of glucagon may be completely counteracted by the activation of GLP1R in a longer term study because of the synergistic insulinoletic action combined with a potential exhaustion of the liver glycogen stores [68]. In this study GLP-1 was infused at 8 pmol/Kg/min resulting in ~4-fold increase in plasma levels of total GLP-1 over baseline (15–23 pmol/l vs. 90–103 pmol/l) while glucagon was infused at 50 mg/kg/min resulting in
~ 25-fold increase in levels vs. baseline (8–11 pmol/l vs. 239–260 pmol/l). It is possible that the infusion of GLP-1 and glucagon does not reflect the relative percent of activation of GLP1R and GCGR in vivo achieved by OXM but rather a relative higher percent activation of GCGR vs. GLP1R. The reduced ability of GLP1R activation to counteract the hyperglycemic effect of glucagon was demonstrated in mice with a set of peptides with increased GCGR affinity relative to GLP1R activation [73]. Another study that potentially shed light on the mechanism of action of OXM demonstrated that selective activation of GCGR increased liver FGF-21 mRNA and FGF-21 plasma concentrations [74,75]. This observation is consistent with increased FGF-21 liver expression observed with an oxyntomodulin analog peptide (GLP1R/GCGR dual agonist), but not with a GLP1R-selective peptide [29]. The authors subsequently found in human obese healthy volunteers that a significant elevation of plasma FGF-21 (Figure 2) was observed after a glucagon challenge (1 mg intramuscularly). Because glucagon receptor activation failed to induce weight loss in mice with genetic deletion of FGF-21, it was suggested that FGF-21 mediates at least part of the effects of glucagon on body weight [74]. Stimulation of plasma FGF-21 by glucagon was also reported in rodents and humans with Type 1 diabetes [76]. Chronic administration of the FGF-21 analog LY2405319 reduced body weight, triglycerides and improved glucose metabolism in diabetic non-human primates and humans [77,78] in association with dose-dependent elevation of plasma adiponectin [78]. Recently adiponectin was suggested as a mediator of the glucose lowering and insulin sensitizing effects of FGF-21 in mice possibly via regulation of PPAR γ [79–81]. Therefore, it is possible that FGF-21 and adiponectin are involved in the metabolic effects of oxyntomodulin. While the studies were performed at pharmacological doses of glucagon, a critical physiological relevance of glucagon in the regulation of body weight and lipid metabolism was established by Engel and colleagues [82]. They found after a 12-week treatment of T2DM patients with GCGR small molecule antagonist dose-dependent elevations in body weight, cholesterol and triglycerides [82]. Thus, oxyntomodulin or simultaneous activation of GLP1R and GCGR should increase plasma concentrations of FGF-21 and adiponectin in humans (Figure 2). In addition, GLP1R/GCGR dual agonism may be involved in the control of expression and release of many protein factors from adipose tissue through FGF-21 control of PPARγ, not just adiponectin. In support of these data, during a 4-week weight loss study performed with OXM [38] adiponectin was significantly increased in the treatment group. Because adiponectin is reduced in obesity and T2DM, GLP1R/GCGR dual agonism may increase or restore the adiponectin deficiency observed in mice and humans as seen following administration of the FGF-21 analog, LY2405319 [66]. Despite the anorectic effects during chronic dosing of OXM in humans [38], there is insufficient evidence to establish whether the chronic body-lowering effect of OXM involves increase in energy expenditure and if FGF-21, adiponectin or other downstream mediators contribute to these effects in humans.

Ghrelin has been shown to inhibit insulin secretion in rodents and humans [83], so ghrelin suppression observed during simultaneous infusion of GLP-1 and glucagon [56], and acute infusion of OXM [37] may be jointly involved with FGF-21 [74] and adiponectin [77–79] in the acute beneficial effects on glucose metabolism [45,48–50]. A potential mechanism that could also be involved in the anti-obesity effects of oxyntomodulin is the direct effect of glucagon on brown adipose tissue associated with the adrenergic GLP1R-dependent stimulation of adipose tissue thermogenesis [84]. In conclusion, OXM acutely improves energy and glucose metabolism in rodents and humans. Despite different affinity for the respective receptors, different pharmacokinetics and tissue distribution, the acute glucoregulatory actions of OXM in mice [45] and human T2DM [50] appear to resemble the results of the simultaneous infusion of glucagon and GLP-1 [68]. A potential limitation that affects the interpretation of the human results is that no data were disclosed supporting engagement of the glucagon receptor in vivo during the weight loss study [38] and the GGI study [50]. Further studies in humans are required to confirm these findings.

8. POTENTIAL ADVERSE EVENTS AND SAFETY CONCERNS

8.1. Gastrointestinal (GI) adverse effects
Nausea, vomiting and diarrhea are commonly reported for GLP1R agonists. They are dose-limiting and in some patients responsible for treatment discontinuation [85]. Delay in gastric emptying may contribute to the GI events reported but the mechanisms underlying these adverse effects are still unclear. The GI tolerability with GLP1R agonists diminish over a period of 4–6 weeks [86]. The nausea associated with pharmacological doses of glucagon is well recognized [87]. Transient mild nausea was reported in 3% of oxyntomodulin self-administered subcutaneous injections for 4 weeks during the body weight loss study conducted in healthy overweight and obese volunteers [38].

8.2. Pancreatitis and pancreatic cancer with incretin-based therapies
A few cases of acute pancreatitis have been reported during treatment with exenatide and other GLP-1 receptor agonists [88]. Acute pancreatitis, including fatal and non-fatal hemorrhagic or necrotizing pancreatitis has been reported in patients prescribed incretin-based agents. The majority of reports are from postmarketing surveillance and it is therefore difficult to determine a true incidence of pancreatitis associated with incretin therapy [88–90]. Of note, glucagon can depress human pancreatic exocrine secretion [91,92]. There are no biomarkers that can prestage pancreatitis in the context of routine clinical surveillance. At the present time, it is unknown whether incretin therapies play a causative role in pancreatitis or whether reported cases reflect the ~2–3 fold increased risk of acute pancreatitis in T2DM [93]. Recently a report showed in pancreases of patients treated with incretin therapies, increased pancreatic mass as well as a proliferation of exocrine cells [94]. This paper triggered several commentaries [95–97] highlighting several issues with the study. Despite the issues raised by investigators, the report will likely prompt reanalysis of clinical data available with these agents and additional post-mortem studies with well-matched cohorts. These results will also likely impact any potential new therapies involving GLP1R activation.

8.3. C-cell hyperplasia and medullary carcinoma of the thyroid
An increased incidence of C-cell hyperplasia and medullary thyroid cancer was reported in rats and mice following continuous GLP1R activation [98]. This effect was demonstrated to be rodent-specific because GLP1R was not detected in monkey and human thyroid and no C-cell hyperplasia was observed in monkeys treated for over 20 months with GLP1R agonists [98]. A meta-analysis of sequential calcitonin measurements in over 5000 patients treated with liraglutide indicated that calcitonin levels remained low throughout the trials, which were from 26 weeks to 2 years duration [99]. Recently GLP-1 receptor immunoreactivity has been reported in five of 15 of the examined cases in about 35% of the total C cells assessed in normal human thyroid tissue [100]. Because glucagon stimulates calcitonin secretion by thyroid C cells [101] long-term consequences of sustained GLP-1 receptor and
has also been demonstrated to produce protein catabolism in human acute infections. All of the aforementioned are complex factors associated with stress, e.g. post-surgery, trauma, burns, and subjects [104]. Glucagon has also been implicated in negative nitrogen enhancement in patients with glucagonoma compared with healthy control demia and whole-body protein breakdown was demonstrated to be Glucagonomas are associated with muscle wasting and hypoaminoacidaemia and whole-body protein breakdown was demonstrated to be enhanced in patients with glucagonoma compared with healthy control subjects [104]. Glucagon has also been implicated in negative nitrogen balance associated with stress, e.g. post-surgery, trauma, burns, and acute infections. All of the aforementioned are complex factors other than glucagon (e.g. other hormones and cytokines) that could be contributing to negative nitrogen balance [105]. Glucagon administration has also been demonstrated to produce protein catabolism in human subjects in the setting of experimentally-induced insulin deficiency [106]. However insulin is a major anabolic hormone and insulin action is not expected to be absent in patients treated with GLP1R/GCGR dual agonism. Nevertheless, the potential for GLP1R/GCGR treatment to cause skeletal muscle loss needs to be appropriately assessed in long term studies.

8.5. Cardiovascular function
Glucagon and GLP-1 have positive inotropic and chronotropic action on the heart [56,107–110]. GLP1R agonists cause a 1–3 mmHg decrease in systolic and diastolic blood pressure, and a 2–4 bpm increase in heart rate [111–120]. Acute administration of 1 mg of glucagon to normal volunteers resulted in increases in heart rate and mean arterial pressure and decreases in systemic vascular resistance [121]. Glucagon causes natriuresis with changes in kidney sodium handling. Enhanced proximal tubular sodium reabsorption and a higher prevalence of hypertension have been associated with the Gly40Ser polymorphism of the glucagon receptor gene resulting in a mutated receptor less responsive to glucagon [122,123]. Consistently, a trend towards blood pressure increase was observed during pharmacological GCGR antagonism in T2DM patients in a 4-week Phase IIa proof-of-concept study [124]. No effects of oxyntomodulin on pulse rate were observed in human studies [37,38,51].

8.5.1. Serum lipids
Serum lipids are another important cardiovascular consideration. Liraglutide is associated with a small increase in HDL-C and modest decreases in LDL-C and triglycerides [111–117]. There is preclinical and clinical evidence that glucagon decreases serum triglyceride, cholesterol, and VLDL cholesterol levels [121]. While initial results suggest a potential cardioprotective effect of GLP-1 based therapies [125] prospective outcome trials are required to confirm these positive findings together with potential risks associated with the increased heart rate in diabetic patients. A body of evidence is beginning to emerge on the long-term CV safety profile of incretin-based therapies. The results of the first trial with saxagliptin met the FDA criteria for non-inferiority over placebo, but did not provide evidence of cardiovascular risk reduction [126]. In summary, these observations suggest the need to carefully assess the impact of GLP1R/GCGR dual agonist treatments on heart rate, systolic and diastolic blood pressure and lipids. Subtle changes in those parameters could alter what is generally viewed as a positive overall cardiac profile of GLP1R-selective agonists.

8.6. Considerations
There is a long list of potential safety concerns associated with pharmacological activation of GLP1R or GCGR. It is important to consider that because of the synergistic metabolic effects of GLP1R and GCGR activation, GLP1R/GCGR dual agonists are expected to require lower plasma concentrations and, therefore, lower receptor activation to elicit its effect on body weight and glucose lowering as demonstrated in rodents and monkeys [29,103,127]. It is difficult to predict whether the documented tolerability, GI issues, the increase in heart rate seen at pharmacological doses of GLP-1 and glucagon and other effects seen with GLP1R agonists or glucagon administration will be observed to the same extent and degree in GLP1R/GCGR dual agonism.

9. THERAPEUTIC POTENTIAL
Gastric bypass is the only approach that produces lasting improvement in glucose metabolism and weight reduction [128]. Among the changes consistently observed following roux-en-Y gastric bypass are exaggerated postprandial increase in OXM, glucagon, PYY and GLP-1 [128,129]. Hence, administration of endogenous gut peptides or more metabolically-stable analog represents a potential long-term therapeutic approach to obesity and diabetes. With the promising results seen with GLP1R agonists [130], the next generations of diabetes drugs will likely focus on the alternate delivery for injectables as illustrated by recent experience with GLP-1 analogs and insulin [131–134] and the combined activation of more than one receptor [135]. Among these, oxyntomodulin is a promising weight-loss and glucose-lowering therapy and appears well-tolerated in human studies reported to date [37,38,50,51]. The clinical utility of OXM is limited, mainly because of its short circulating half-life [52]. Because glucagon and GLP-1 share ~50% amino acid sequence identity (Figure 3), several groups have recently developed GLP11/GCGR dual agonist peptides that are resistant to peptidase degradation [29,103,127,136,137]. Two independent papers reported for the first time the use of GLP1R/GCGR dual agonists as being of enhanced efficacy relative to pure GLP1R agonists in the treatment of rodent obesity, with simultaneous improvement in glycemic control [29,103].

| Peptides | 1  | 10 | 20 | 30 | 40 |
|----------|----|----|----|----|----|
| GLP-1    | HAE GTFT SDV SYYL EG QAAK EF IAWL V K GR |
| Glucagon | HSQ GTFT SDY SKYL DS RR AQDFVQWLMT |
| GIP      | Y AE GTFI SDY SIAM DK IH QQDFVNW L AQKGGKNDWKHNI TQ |

Figure 3: Amino acids sequence alignment of GLP-1, glucagon and glucose-dependent insulinotropic peptide (GIP).
The hyperglycemic risk posed by GCGR has triggered questions about the appropriate ideal ratio of receptor activation. When a PEGylated GLP1R/GCGR dual agonist was given to Glpr\textsuperscript{-/-}\textsuperscript{mice}, the decrease in body weight was no longer associated with improvement in glucose metabolism [103]. Using a spectrum of peptides with different relative receptor selectivity, it was demonstrated that a dual agonist peptide with comparable functional potencies at the GLP1R and GCGR (similar ratio to native OXM) maximizes the weight loss and mitigated the hyperglycemic risk associated with GCGR activation [73]. Recently the translation of these observations was reported to obese, non-diabetic rhesus monkeys, which were treated with a protease-resistant GLP1R/GCGR dual agonist [127]. Daily administration of a lipidated dual agonist at a nearly seven-fold lower dose (3 μg/kg s.c.) to liraglutide (GLP1R-selective agonist, 20 μg/kg s.c.) caused superior weight loss. The same group evaluated the impact on glycemic control and the potential diabetogenic risk of GCGR activation in diabetic rhesus monkeys demonstrating reductions in fasting glucose and improved glucose tolerance in the absence of changes in body weight [127]. These results demonstrate successful translation of the superior pharmacology of GLP1R/GCGR dual agonists from rodents to non-human primates deepening our belief that this approach constitutes a promising new mechanism in the treatment of T2DM.

10. CONCLUSION

Activation of GLP1R and GCGR with chimeric peptides and in the future orally available GLP1R/GCGR dual agonists is a conceivable option to achieve improved therapeutic goals. It is also conceivable to stimulate endogenous secretion of oxyntomodulin from intestinal L-cells and leveraging the similarity of glucagon-based peptides to activate GLP1R, GCGR and GIP receptors (Figure 3) [138,139] and other family B GPCRs. Nevertheless, it will be critical to deepen our understanding of the mechanisms of action and how structurally related peptides like GLP-1 and glucagon interact with their respective receptors [140,141]. Important considerations for the success of future GLP-1 based therapies will not only be efficacy but also a good safety profile and compliance. Future injectable therapies will need to significantly lower glucose and exceed the benefits of current GLP1R-selective agonists in addition to possessing superior efficacy on body weight and lipid metabolism. Bariatric surgery lowers body weight and often normalizes HbA1c in T2D patients [128,129] showing that although other mechanisms besides glucagon–peptides may be involved, the enhanced therapeutic goal is achievable. Observing a cardiovascular protective effect on outcome trials would also be ideal. Safety concerns attributed to GLP-1-based therapies such as risk of pancreatitis will need to be explored and may constitute a major differentiation factor. For example, for GLP1R/GCGR dual agonists, glucagon has been shown to depress human pancreatic exocrine secretion and was proposed as a possible treatment for acute pancreatitis [91,92]. GIPR activation does not appear to reduce gastric emptying in healthy humans although it may be involved in distal bowel motility [142]. Therefore other combinations like GLP1R/GIPR dual agonists or GLP1R/GCGR/GIPR triple agonists could possibly offer enhanced efficacy with a superior tolerability profile. Identifying new transformational approaches to controlling T2D that free patients from frequent self-injections resulting in enhanced therapeutic compliance and control is critical. An example is the approach of Intarcia Therapeutics which optimizes GLP-1 therapy with a once- or twice-yearly dosing of ITCA 650, a device that allows continued subcutaneous delivery of exenatide. A potential limitation of this approach is the ambulatory surgery required for the implant and replacement of this device [143]. Nevertheless, it may help enhance adherence not only in diabetic patients but in diabetic patients with neurodegenerative diseases [144]. Despite recent innovations for injectables, the oral route of administration remains the most widely accepted [145] and will need to be considered together with the safety and efficacy profile for the success of new GLP-1 based therapies. Future uses of these drugs might also include the treatment of obesity [146] as well as adjunctive treatment of type 1 diabetes mellitus in combination with insulin. Addition of liraglutide was shown to reduce the insulin dose required to maintain glucose control in T1D patients [147] potentially lowering the risk of hypoglycemia. The fear of iatrogenic hypoglycemia remains a major obstacle for appropriate glucose control in patients with both Type 1 and advanced Type 2 diabetes mellitus where the counter-regulatory response is impaired especially following recurrent hypoglycemic episodes [148,149]. Sustained GCGR activation in conjunction with GLP1R activation may further reduce the hypoglycemic risk and weight gain during intensive insulin therapy and, although more research is needed, the potential exists to uncover alternate and viable treatment options for individuals living with diabetes.

CONFLICT OF INTEREST

The author is an employee of Janssen R&D, pharmaceutical companies of Johnson & Johnson.

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