REVIEW

Unraveling oxyntomodulin, GLP1's enigmatic brother

Alessandro Pocai

Diabetes and Endocrinology, Merck Research Laboratories, Merck Sharp and Dohme Corp., 126 East Lincoln Avenue, Rahway, New Jersey 07065, USA
(Correspondence should be addressed to A Pocai; Email: alessandro_pocai@merck.com)

Abstract

Oxyntomodulin (OXM) is a peptide secreted from the L cells of the gut following nutrient ingestion. OXM is a dual agonist of the glucagon-like peptide-1 receptor (GLP1R) and the glucagon receptor (GCGR) combining the effects of GLP1 and glucagon to act as a potentially more effective treatment for obesity than GLP1R agonists. Injections of OXM in humans cause a significant reduction in weight and appetite, as well as an increase in energy expenditure. Activation of GCGR is classically associated with an elevation in glucose levels, which would be deleterious in patients with T2DM, but the antidiabetic properties of GLP1R agonism would be expected to counteract this effect. Indeed, OXM administration improved glucose tolerance in diet-induced obese mice. Thus, dual agonists of the GCGR and GLP1R represent a new therapeutic approach for diabetes and obesity with the potential for enhanced weight loss and improvement in glycemic control beyond those of GLP1R agonists.
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Introduction

As the prevalence of type 2 diabetes increases, there is a medical need for additional antihyperglycemic agents that offer improved efficacy in glycemic control and tolerability. Obesity is an important risk factor for a number of debilitating chronic conditions such as T2DM, dyslipidemia, and hypertension (Bray 2004). However, several T2DM therapies have been associated with weight gain, most profoundly for the sulfonylureas, meglitinides, and thiazolidinediones (TZDs) as well as for insulin. Recent work on understanding the physiological function of proglucagon-derived peptides has renewed interest in glucagon-based therapeutics. One of these peptides is glucagon-like peptide-1 (GLP1), which is secreted from the L cells of the gastrointestinal tract and lowers blood glucose levels primarily by promoting insulin secretion and by inhibiting glucagon secretion (Holst 2000). GLP1 has been found to decrease food intake and inhibit gastric emptying (Holst 2000, D'Alessio 2008). GLP1 is rapidly inactivated by dipeptidyl peptidase-4 (DPP4) and its renal clearance is relatively fast (Field *et al*. 2009). Accordingly, new drugs based on GLP1 receptor (GLP1R) agonism and DPP4 inhibition have been approved for the treatment of type 2 diabetes, but the magnitude of weight loss at tolerated doses is modest (Amori *et al*. 2007). Nevertheless, protease-resistant GLP1R agonists (Drucker *et al*. 2010, Htike *et al*. 2012, Nauck 2012) represent a new class of antihyperglycemic agents that reduce body weight (D'Alessio 2008, Vilsboll *et al*. 2012) and are currently being tested for the treatment of obesity (Astrup *et al*. 2012).

The preproglucagon family: historical overview and tissue-specific posttranslational processing

Oxyntomodulin (OXM) is a 37-amino acid peptide secreted in proportion to nutrient ingestion (Ghatei *et al*. 1983, Le Quellec *et al*. 1992, Holst 1997, Drucker 2005) comprising the entire 29-amino acid sequence of glucagon, with an 8-amino acid carboxy-terminal extension that results from the processing of the preproglucagon precursor (Fig. 1; Campos *et al*. 1994, Larsen *et al*. 1997). The processing of proglucagon is tissue specific, producing from a single protein different hormones depending on the tissue considered. In pancreatic α cells, prohormone convertase 2 (PC2) generates predominantly glucagon (Rouille *et al*. 1994, Kieffer & Habener 1999, Furuta *et al*. 2001), whereas in intestinal L cells present in the jejunum, ileum, and colon, PC 1/3 predominantly produces glicentin, OXM, GLP1, and GLP2 (Ghatei *et al*. 1983, Le Quellec *et al*. 1992, Holst 1997, Drucker 2005, Brubaker 2012, Habib *et al*. 2012). Similar processing is also thought to occur in the same neurons in the nucleus of the solitary tract (NTS) in the hindbrain (Holst 2007; Fig. 1).

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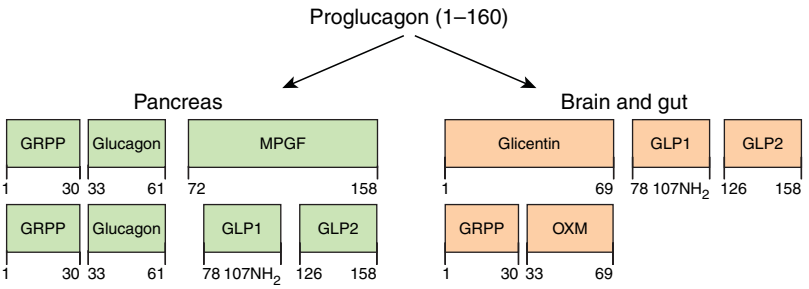

Figure 1 Tissue-specific processing of proglucagon. Differential posttranslational processing of proglucagon in the pancreas and in the gut and brain. Preproglucagon is proteolytically cleaved in a tissue-specific manner by prohormone convertases 1 and 2. The numbers in the figure indicate amino acid positions in the 160-amino acid proglucagon sequence. In the pancreas, processing yields the glucagon sequence, whereas the region containing the GLP1 and GLP2 peptides is secreted as a single inactive fusion called major proglucagon fragment (MPGF) or GLP1 and GLP2. Posttranslational processing in the gut and brain results in the secretion of GLP1 and GLP2, while the glucagon sequence remains in a larger peptide, glicentin or glicentin-related pancreatic peptide (GRPP), and oxyntomodulin.

Sutherland & De Duve (1948) described for the first time glucagon-like substances in extracts of intestinal mucosa as identified using a bioassay (Holst 1983). The description of a glucagon RIA by Unger *et al.* (1959), (1961) and (1962) made it possible to confirm that the intestinal extracts contained peptides cross-reacting in the RIA (gut 'glucagon-like immunoreactivity', GLI). The first evidence of the existence of OXM was generated in 1968 when it was discovered that the gut GLI consisted of at least two peptides. Two distinct moieties with GLI were secreted in response to an oral glucose load (Unger *et al.* 1968, Valverde *et al.* 1968). One with a C-terminal octapeptide extension (SP-1, spacer peptide-1, or KA-8 or IP-1, intervening peptide) was named OXM (Bataille *et al.* 1981*b*, Dubrasquet *et al.* 1982) for its ability to modulate gastric acid secretion in gastric oxyntic glands (Bataille *et al.* 1981*a*,*b*,*c*, 1982), and the other with the same C-terminal extension plus an N-terminal extension of 30 amino acids, was named glicentin (Sundby *et al.* 1976, Thim & Moody 1981, Holst 1982). Larsson *et al.* (1975) using immunohistochemistry and radioimmunoanalysis showed in 1975 that disseminated cells that predominate in the ileum and colon intestinal mucosa store gut-type glucagon, reporting for the first time the spatial tissue distribution of OXM in the gut. The distribution of glucagon-related peptides in the human gastrointestinal mucosa was later described by Baldissera & Holst (1984).

Receptors and post-receptor pathways activated by OXM

OXM binds to and is a full ~ equipotent agonist of the human GLP1R and glucagon receptor (GCGR)-mediated cAMP accumulation, although with reduced affinity compared with the cognate agonists GLP1 and glucagon (Bataille *et al.* 1982, Baldissera *et al.* 1988, Gros *et al.* 1993, Schepp *et al.* 1996, Baggio *et al.* 2004, Jorgensen *et al.* 2007, Pocai *et al.* 2009, Kosinski *et al.* 2012). Despite being a full agonist at the human GLP1R, OXM was found to be a full agonist in recruiting β-arrestin 2 to the GCGR, but partial agonists in recruiting β-arrestin (β-arrestin 1 and β-arrestin 2) and G-protein-coupled receptor (GPCR) kinase 2 to the GLP1R. Consistent with the properties of a partial agonist, OXM was a functional antagonist of GLP1-induced agonist response in β-arrestin 2 recruitment. It has been suggested that OXM is a GLP1R-biased agonist relative to GLP1 having less preference toward cAMP signaling relative to phosphorylation of ERK1/2, but similar preference for cAMP relative to Ca^{2+} (Jorgensen *et al.* 2007). These findings imply that the GLP1R-mediated *in vivo* effects of OXM could differ from that of GLP1 (Koole *et al.* 2010). Currently, no data are available on whether OXM interact with other family B GPCRs such as GLP2 and glucose-dependent insulinotropic peptide (GIP) receptors. Peripheral administration of OXM results in increased c-Fos in the arcuate nucleus (ARC), but not in the brainstem region (Dakin *et al.* 2004), and using manganese-enhanced magnetic resonance to follow the pattern of neuronal activation, OXM and GLP1 result in activation of different hypothalamic pathways (Chaudhri *et al.* 2006, Parkinson *et al.* 2009). While these differences may be simply due to a different brain penetration, it is plausible that the engagement of additional receptor(s) and/or the above reported difference on GLP1R signaling pathways may explain the differences.

OXM and body weight

OXM causes weight loss in obese patients (Wynne *et al.* 2005) and rodents (Dakin *et al.* 2001, Baggio *et al.* 2004) via

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suppression of food intake and increases in energy expenditure (Baggio *et al.* 2004, Wynne *et al.* 2006). OXM is a dual agonist at the GLP1R and GCGR *in vitro* (Baldissera *et al.* 1988, Gros *et al.* 1993, Pocai *et al.* 2009). The anorectic effects of central administrations of GLP1 and OXM are abolished by co-administration of the GLP1R antagonist, exendin(9–39), and are not observed in *Glp1r*$^{-/-}$ mice, suggesting that the central effect of OXM is mediated by the GLP1R (Turton *et al.* 1996, Dakin *et al.* 2001, Baggio *et al.* 2004, Sowden *et al.* 2007, Wynne *et al.* 2010). While data obtained with the truncated lizard peptide exendin(9–39) needs to be carefully evaluated as it was shown to function either as a GLP1 and GLP2 receptor antagonist and to displace GIP binding *in vitro* (Wheeler *et al.* 1995, Brubaker *et al.* 1997, Tang-Christensen *et al.* 2000), the lack of OXM efficacy seen in *Glp1r*$^{-/-}$ mice demonstrated that the initial anorectic effect of OXM is mediated solely by activation of the GLP1R (Baggio *et al.* 2004, Sowden *et al.* 2007). However, other acute effects of OXM, including stimulation of heart rate and energy expenditure, appear to be independent of GLP1R, suggesting that OXM has both GLP1R-dependent and independent effects *in vivo* (Baggio *et al.* 2004, Sowden *et al.* 2007).

These data led several investigators to hypothesize that the differential effect of OXM vs GLP1 could be mediated by activation of the GCGR or an as of yet unidentified OXM-specific receptor. Recently, several publications (Du *et al.* 2012, Kosinski *et al.* 2012) expanded the initial findings on the mechanism of action of OXM and demonstrated that OXM has glycogenolytic properties in perfused mice liver showing that OXM can functionally activate the GCGR (Kosinski *et al.* 2012). Du *et al.* (2012) using a GLP1R agonist peptide with a mutation of Gln (Q) → Glu (E) (OXMQ3E) in position 3 that dials out activity on GCGR (Santoprete *et al.* 2011; Fig. 2), demonstrated that OXM, but not OXMQ3E, stimulated liver ketogenesis in wild-type mice. A similar effect was observed in *Glp1r*$^{-/-}$ but not in *Gcgr*$^{-/-}$ mice demonstrating that this effect of OXM is mediated by GCGR activation (Du *et al.* 2012). When equimolar doses of OXM (GLP1R/GCGR dual agonist) and OXMQ3E (selective GLP1 agonist) were infused in obese mice, OXM exerted superior weight loss and lipid lowering with comparable glucose lowering to OXMQ3E (Kosinski *et al.* 2012). Moreover, chronic infusion of OXM in *Glp1r*$^{-/-}$ mice retained some of the body weight effect observed in lean wild-type mice, and pharmacological blockade of the GCGR during OXM infusion demonstrated that the additional body weight lowering observed with OXM vs OXMQ3E is mediated by activation of the GCGR. This study showed the involvement of the GCGR together with GLP1R activation to the body weight lowering effect of OXM, but it did not completely dismiss the potential contribution of an OXM-specific receptor as minor weight loss was observed in the group treated with a small molecule GCGR antagonist alone (Kosinski *et al.* 2012). The superior weight loss efficacy of OXM vs GLP1R agonism is consistent with previous research on glucagon and energy homeostasis in humans and rodents. Repeated administration of glucagon was first shown to inhibit food intake in man over 50 years ago (Schulman *et al.* 1957), and aside from its well-known hyperglycemic action, glucagon increases thermogenesis, satiety, lipolysis, fatty acid oxidation, and ketogenesis (Salter 1960, Salter *et al.* 1960, Penick & Hinkle 1961, Langhans *et al.* 1982, Habegger *et al.* 2010, Jones *et al.* 2012). A critical physiological role of glucagon in the maintenance of whole-body energy homeostasis was supported by a recent study in T2D patients where a dose-dependent increase in body weight was observed following pharmacological blockade of the GCGR (Engel *et al.* 2011).

	1.....11 ..... 21.....31
GLP1	HAEGTFTSDVS SYLEGQAAK E F IAWLVKGR
Glucagon	HSQGTFYSDYS KYLDSRRAQ DFVQWLMNT
Glicentin	RSLQDTEEKSRSFSASAADPLSDPDQMNEDKR HSQGTFTSDYS KYLDSRRAQ DFVQWLMNTKRNRNNIA
OXM	HSQGTFTSDYS KYLDSRRAQ DFVQWLMNT KRNRNNIA
OXMQ3E	HSEGTFTSDYS KYLDSRRAQ DFVQWLMNT KRNRNNIA

Figure 2 Sequence comparisons of GLP1(7–36) amide, glucagon(1–29), glicentin, OXM, and OXMQ3E.

OXM and glucose metabolism

Studies have suggested that OXM may play a role in glucose homeostasis. Chronic treatment with OXM results in superior weight-lowering and comparable antihyperglycemic effect to a GLP1R-selective agonist (Kosinski *et al.* 2012). This is likely achieved through body weight reduction due to the causal link between obesity and type 2 diabetes (Karra & Batteram 2010) as well as direct enhancement of glucose-dependent insulin secretion (Maida *et al.* 2008, Parlevliet *et al.* 2008, Du *et al.* 2012). Activation of GCGR is associated with an elevation in glucose levels but the simultaneous agonism at the GLP1R would be expected to counteract this effect. Acute treatment with OXM improves glucose tolerance during a glucose challenge in mice (Maida *et al.* 2008, Parlevliet *et al.* 2008). Moreover, OXM administration improved glucose intolerance by enhancing glucose disposal during a hyperinsulinemic clamp study performed in diet-induced insulin-resistant mice (Parlevliet *et al.* 2008). It has been proposed that following a single injection, OXM acts solely via GLP1R to modulate glucose homeostasis (Maida *et al.* 2008). However, OXM was reported to increase hepatic glucose production during a euglycemic–hyperinsulinemic clamp performed in diet-induced obese mice, suggesting activation of the hepatic GCGR *in vivo* (Parlevliet *et al.* 2008). Recently, it was demonstrated that while acute treatment with OXM improves glucose metabolism during a glucose tolerance test and during a hyperglycemic clamp in mice, a matched pair peptide without GCGR activity (OXMQ3E) (Fig. 2) exerted better glucose-lowering properties compared with OXM administration (Du *et al.* 2012). The same authors

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showed decreased glucose tolerance in OXM-infused compared with vehicle-infused *Glp1r*$^{-/-}$ mice. The lack of effect observed following a single i.p. injection of OXM during a glucose tolerance test in *Glp1r*$^{-/-}$ mice (Maida *et al.* 2008) may be explained by the fact that *Glp1r*$^{-/-}$ mice are glucose intolerant and resistant to diet-induced obesity; hence, the acute glucoregulatory effect of a single injection of OXM could be confounded by compensatory mechanisms associated with chronic deletion of the GLP1R (Flamez *et al.* 1999). To further strengthen these data, hyperglycemic clamps performed in *Gcgr*$^{-/-}$ mice showed a similar effect of OXM and OXMQ3E infusion on glucose metabolism in the absence of a functional GCGR. This study demonstrated that simultaneous activation of the GLP1R counteracts the hyperglycemic effect of glucagon *in vivo*. The glucose-lowering effect of OXM is mostly mediated by GLP1R activation and activation of the GCGR appears to limit the acute antihyperglycemic efficacy of OXM while contributing to the insulinotropic properties of OXM (Du *et al.* 2012). Glucagon has been reported to increase glucose levels following i.c.v. administration in rats (Marubashi *et al.* 1985, Amir 1986). However, Mighiu *et al.* (2012) recently demonstrated that intrahypothalamic glucagon suppresses hepatic glucose production and counteracts the direct hepatic stimulatory effect of circulating glucagon on liver glucose production in rodents during a pancreatic clamp. Therefore, activation of the GCGR in discrete CNS areas may contribute to the improvement of whole-body glucose metabolism in animals treated with OXM.

Other actions of OXM

Gastrointestinal effects

OXM decreases gastric acid and pancreatic exocrine secretion (Bataille *et al.* 1981*a*, Dubrasquet *et al.* 1982, Ghatei *et al.* 1983, Biedzinski *et al.* 1987, Schjoldager *et al.* 1989, Le Quellec *et al.* 1992, Anini *et al.* 2000) and increases intestinal glucose uptake in preclinical species (Collie *et al.* 1997). Acute administration of OXM does not decrease gastric emptying in mice (Maida *et al.* 2008) while i.v. infusion of OXM inhibits gastric emptying in humans (Schjoldager *et al.* 1989). GLP1 is thought to reduce postprandial glucose excursion primarily via deceleration of gastric emptying in rodents and humans (Wettergren *et al.* 1993, Willms *et al.* 1996), although this effect seems to desensitize after multiple administrations (Meier *et al.* 2005, Nauck *et al.* 2011) and glucagon has also been reported to decrease gastric emptying (Habegger *et al.* 2010). Further studies are required to confirm or reconcile these divergent findings on gastric emptying in mice and humans potentially involved in the effect of OXM on the regulation of glucose and energy metabolism (Fig. 3).

Pancreatic effects

OXM has been reported to decrease pancreatic secretion through the nervous system in rats (Anini *et al.* 2000) and stimulate the endocrine pancreas to secrete insulin, somatostatin, and glucagon (Baldissera *et al.* 1988, Schjoldager *et al.* 1988,

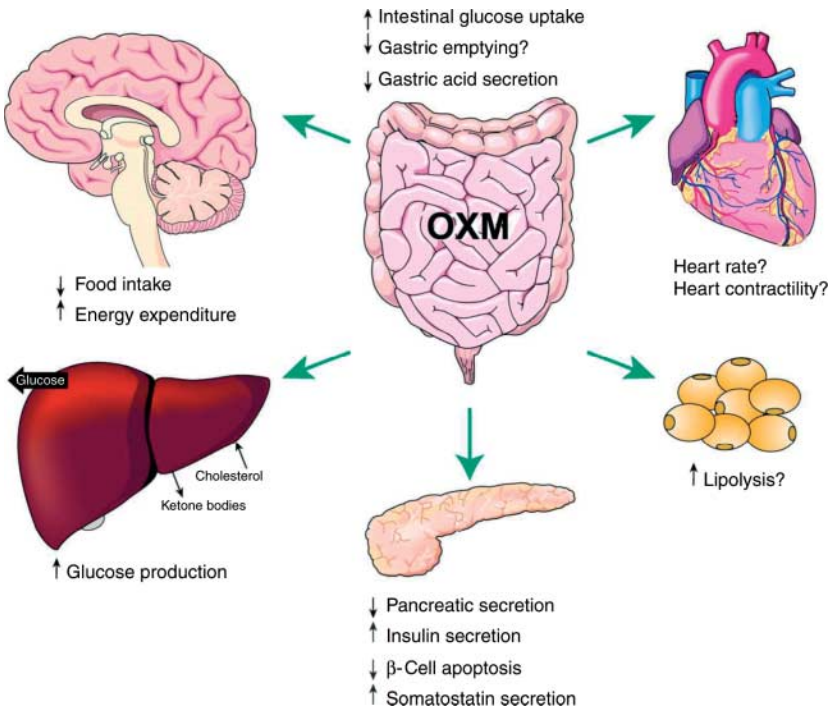

Figure 3 Reported effects of oxyntomodulin.

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Maida *et al.* 2008, Parlevliet *et al.* 2008, Du *et al.* 2012). OXM is capable of stimulating insulin secretion in a glucose-dependent manner. This effect is likely mediated through sensory nerves expressing the GLP1R and direct activation of the β-cells GLP1R and GCGR (Drucker *et al.* 1987, Ahren 2004, Vahl *et al.* 2007, Maida *et al.* 2008, Du *et al.* 2012). It is not known whether the GCGR is involved in OXM-mediated insulin secretion via sensory pathways. Furthermore, OXM reduces β-cell apoptosis and increases pancreatic insulin content in diabetic mice treated with streptozotocin, an effect mediated at least in part via a direct GLP1R-dependent mechanism (Maida *et al.* 2008). Recently, the GLP1R-mediated proliferative effect on the β-cell has been demonstrated to require a signaling complex GLP1R/β-arrestin 1/c-Src in INS832/13 cells (Talbot *et al.* 2012; Fig. 3).

Cardiovascular effects

Glucagon and GLP1 have positive inotropic and chronotropic actions on the heart (Farah & Tuttle 1960, Buse *et al.* 1973, Gonzalez-Munoz *et al.* 2008, Grieve *et al.* 2009). OXM administration increased heart rate in wild-type mice (Sowden *et al.* 2007). Involvement of the GCGR in the effects of OXM on mouse heart is consistent with the increased heart rate observed in *Glp1r*$^{-/-}$ mice (Sowden *et al.* 2007). Further studies are required to investigate the effect of OXM on the heart, as no increase in heart rate was detected following administration of OXM in rat and humans (Wynne *et al.* 2005, 2006, Sowden *et al.* 2007). The GLP1R/GCGR dual agonist ZP2495 has been shown to increase glucose oxidation and glycolytic rates in insulin-resistant hearts of obese insulin-resistant rats but, unlike glucagon, it did not compromise the energetic state of the hearts and GLP1 agonism had no effect on cardiac metabolism (Axelsen *et al.* 2012). The authors suggested that GLP1R/GCGR dual agonists may have inotropic and energy-preserving effects in insulin-resistant hearts and that these effects could be beneficial for the treatment of heart failure or cardiogenic shock in subjects with insulin resistance (Axelsen *et al.* 2012).

Renal effects

GLP1 agonists and DPP4 inhibitors cause moderate blood pressure lowering and lipid lowering in humans that may contribute to the reported cardiovascular benefits of this class of antidiabetic drugs (Mega *et al.* 2011, Liu *et al.* 2012, Panchapakesan *et al.* 2013). 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 GCGR gene resulting in a mutated receptor less responsive to glucagon (Strazzullo *et al.* 2001, Barbato *et al.* 2003). Consistently, a trend toward blood pressure increase was observed during pharmacological GCGR antagonism in T2D patients in a 4-week Phase IIa proof-of-concept study (Ruddy *et al.* 2011). No data so far have been published on the renal effects of OXM.

Neuroprotective effects

In preclinical studies, GLP1 agonists and DPP4 inhibitors (in association with GLP1 elevation) have been shown to counteract memory impairment, protect neurons from oxidative stress, and reduce plaque formation and the chronic inflammation response in the brains of mouse models of Alzheimer's disease, Parkinson's disease, and other degenerative diseases (Wu *et al.* 2003, D'Amico *et al.* 2010, Holscher 2012). GLP1R agonists are currently explored for the treatment of neurodegeneration (Mega *et al.* 2011, Liu *et al.* 2012, Panchapakesan *et al.* 2013), and glucagon has been recently reported to preserve neurological function following brain trauma in diabetic rats (Abu Fanne *et al.* 2011). No published reports so far have explored the effect of OXM or GLP1R/GCGR dual agonists on neuroprotection. The above studies suggest that simultaneous activation of the GLP1R and GCGR could result in potential beneficial effects in neurodegenerative disorders.

OXM, physiological or pharmacological role?

Because of the structural similarities of the proglucagon products, investigating the endogenous levels of OXM is difficult as 'OXM-like immunoreactivity' (OLI) has been estimated mostly using a two-step subtraction RIA or a RIA using an OXM C-terminal specific antibody (Blache *et al.* 1988). In the subtraction RIA, one assay is used to measure the concentration of total glucagon, using antibodies to the nonterminal epitopes of glucagon, which cross-react with glucagon, glicentin, and OXM (Fig. 2). A second assay is used to measure glucagon using antibodies to the exposed C-terminal of glucagon. To estimate OLI, the level of pancreatic glucagon is subtracted from the level of total glucagon (Kervran *et al.* 1987).

Using the OXM C-terminal octapeptide-specific antibody (Blache *et al.* 1988, Le Quellec *et al.* 1992), it was shown that, following i.d. administration of a meal, OLI raised in anesthetized rat plasma from 20·1±2·7 to 176·1±12·2 pmol/l at 30 min (Anini *et al.* 1999). Using the same RIA, Le Quellec *et al.* (1998) determined that fasting and meal-stimulated levels in healthy children were 71±10 and 130±26 pmol/l respectively and that the physiological 24-h OLI profile in human plasma shows a diurnal variation independent of food intake; the lowest levels were found in the early morning (~13·5 pmol/l) and the highest levels were found in the evening (~30·8 pmol/l) (Le Quellec *et al.* 1992). More recently, the subtraction RIA (Ghatei *et al.* 1983) has been used to estimate the OLI concentration in humans treated with OXM (Wynne *et al.* 2005). S.c. OXM (400 nmol preprandially) administered three times daily in overweight and obese subjects over a 4-week period resulted in

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a significant weight loss of 2·3 kg (Wynne *et al.* 2005). The endogenous fasting level of OLI was found to be 97·4±5·5 pmol/l, increasing to 116·5±10·4 pmol/l after a meal. OLI increased tenfold (972±165 pmol/l) 30 min after self-administration of OXM. These levels are similar to those found in gastrointestinal disease such as the levels reported in patients with tropical malabsorption (tropical sprue, approximately tenfold elevation vs normal patients) (Besterman *et al.* 1979), patients who have had small intestinal resection (Besterman *et al.* 1982), and following jejuneal ileal bypass surgery (Holst *et al.* 1979, Sarson *et al.* 1981). The same authors using a similar experimental design and OXM administration demonstrated that 4-day s.c. self-administration of preprandial OXM (400 nmol) three times daily increased energy expenditure and decreased energy intake in overweight and obese volunteers (Wynne *et al.* 2006). The baseline plasma OXM levels were 41±7 pmol/l increasing to 63±16 pmol/l postprandially. When participants self-administered OXM, levels increased to 658±85 pmol/l immediately before the study meal (Wynne *et al.* 2006). Holst's group using a different subtraction RIA to obtain OLI concentrations (Holst *et al.* 1976) reported fasting and postprandial plasma levels of 20–30 pmol/l (Falken *et al.* 2011).

The issues with the accurate quantification of OXM remain a confounder as a proportion of the OLI is represented by glicentin and different assays result in different readouts. Recently, Halquist *et al.* (2012) reported a two-dimensional reversed phase ion pair chromatography–tandem mass spectrometry approach for the analysis of OXM in rat plasma. This LC–MS method may provide new avenues of data that may not have been seen with other method platforms.

Based on the data available, it is unclear what the absolute values of OXM/OLI are in plasma during fasting and following a meal. It is, therefore, difficult to make conclusions about a potential physiological role of OXM. OXM shows similar functional potencies (EC_{50}, cAMP production) at the GLP1R and GCGR (Baldissera *et al.* 1988, Gros *et al.* 1993, Holst 2000, Pocai *et al.* 2009, Kosinski *et al.* 2012) at ∼1–2 orders of magnitude higher than the plasma levels of OXM/OLI reported in postprandial state and then only approach EC_{50} plasma levels when OXM is exogenously administered (Wynne *et al.* 2005, 2006). Based only on peripheral plasma OXM levels, it is unlikely that the endogenous levels of OXM result in physiological effects. More sophisticated technologies measuring the levels of OXM in critical sites such as the vagus or discrete brain-specific areas involved in the regulation of energy homeostasis are not readily available and are required to make any further conclusion. In these sites, the levels of OXM are anticipated to be higher in the postprandial state. Similarly, animal (Vahl *et al.* 2007) and human (Plamboeck *et al.* 2012) studies have demonstrated that an intact vagal innervation is important for the effect of GLP1 in the maintenance of glucose homeostasis. GLP1 is a substrate of DPP4 and is rapidly metabolized to GLP1 (9–37) or GLP1(9–36)NH2. DPP4, among other tissues and cell types, is found on the surface of endothelial cells of capillaries in the intestinal mucosa adjacent to the sites of GLP1 secretion (Hansen *et al.* 1999, Baggio & Drucker 2007). Consequently, <25% of newly secreted GLP1 enters the portal vein in intact form and only ∼10–15% reaches the systemic circulation in the intact insulinotropic form where DPP4 also exists as a soluble form (Pridal *et al.* 1996, Mentlein 1999). OXM has a short half-life in circulation of ∼12 min in humans (Schjoldager *et al.* 1988) and ∼6 min in the rat (Kervran *et al.* 1990). It is removed by renal clearance and is a substrate of DPP4 (Zhu *et al.* 2003). Evidence of the involvement of DPP4 in the breakdown of OXM *in vivo* came from a study in which co-administration of OXM and a DPP4 inhibitor resulted in a greater reduction of food intake (Druce & Bloom 2006). The midsection of OXM may also be a target for degradative enzymes such as the ectopeptidases (Hupe-Sodmann *et al.* 1997) and neutral endopeptidase 24.11 (NEP-24.11, neprilysin) (Hupe-Sodmann *et al.* 1995, Deacon 2005, Plamboeck *et al.* 2005). This observation raised concerns regarding the relevance of portal GLP1 or other peptide concentrations in regulating glucose homeostasis and other pharmacodynamic effects and represents a potential limitation in the majority of studies that rely only on measurement of systemic levels of native peptides (Vahl *et al.* 2007).

Therefore, it is possible that the circulating active levels of OXM reflect, as in the case of GLP1, just a small portion of the secreted product and that the concentration at the site of secretion in the gut or brain is sufficient to elicit physiological responses (Kervran *et al.* 1990). It is also possible that additional receptors are involved in the action of OXM or that OXM acts synergistically when secreted together with other peptides (Field *et al.* 2010) recapitulating the post-Roux-en-Y gastric bypass situation (Ashrafian *et al.* 2011, Jorgensen *et al.* 2012). The understanding of the role of OXM as a potential physiological regulator of appetite and energy expenditure would be strengthened by the identification of a specific OXM receptor, by studies employing specific OXM antagonists or immuno-neutralizing antibodies that block the effects of OXM, but not glucagon or GLP1, or with genetic mouse models.

Therapeutic potential

The only treatments to date that produce lasting weight reduction are gastric banding and gastric bypass surgery, although the associated risk and costs limit their use. Patients that undergo gastric bypass have alterations of several signals that may contribute to their reduced appetite and enhanced glucose homeostasis. Among the changes consistently observed following RYGB are an exaggerated postprandial increase in OXM, glucagon, PYY, and GLP1 (Chandarana & Batterham 2012). Administration of endogenous gut peptides or more metabolically stable analog represents a potential long-term therapeutic approach to obesity and diabetes. OXM administered three times a day preprandially was demonstrated to reduce body weight in humans

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(Wynne *et al*. 2005) and preclinical data suggest that OXM may have glucose-lowering properties (Maida *et al*. 2008, Parlevliet *et al*. 2008). However, the clinical utility of OXM is limited, mainly because of its short circulating half-life (Schjoldager *et al*. 1988). Repeated daily doses of large amounts of peptide would be required to elicit its effect, entailing a treatment regimen inconvenient for patients and not economically viable. Because glucagon and GLP1 share ~50% amino acid sequence identity (Fig. 2), several groups have recently developed protease-resistant GLP1R/GCGR dual agonist peptides that are resistant to peptidase degradation (Day *et al*. 2009, Kerr *et al*. 2010, Liu *et al*. 2010, Santoprete *et al*. 2011). Two independent papers reported the use of GLP1R/GCGR co-agonists as being of enhanced efficacy relative to pure GLP1R agonists in the treatment of rodent obesity, with simultaneous improvement in glycemic control (Day *et al*. 2009, Pocai *et al*. 2009). Two DPP4-resistant OXM analogs have been tested in obese mice to compare the effects of dual agonism relative to activation of the GLP1R (Pocai *et al*. 2009). One analog, being a dual agonist at the GLP1R and GCGR (DualAG), is the OXM peptide with a cholesterol group attached to the C-terminal end. The alternative analog is a GLP1R-selective analog (GLPAG) with an equal affinity for the GLP1R but no significant activity at the GCGR due to a mutation from glutamine to glutamate that abolished the interaction with the GCGR (Santoprete *et al*. 2011). Obese mice administered with DualAG had superior weight loss and lipid lowering compared with the GLP1R-selective agonist (Pocai *et al*. 2009). Another dual GLP1R and GCGR agonist strategy involved screening a series of chimeric DPP4-resistant PEGylated peptides. The chimeric peptide was optimized to decrease food intake, reduce body weight, and increase GLP1 activity to neutralize the hyperglycemic effects of glucagon with weekly s.c. injections to diet-induced obese mice (Day *et al*. 2009). The enhanced weight loss observed with GLP1R/GCGR dual agonists (Day *et al*. 2009, Pocai *et al*. 2009, Kosinski *et al*. 2012) has triggered important questions about the ideal ratio of receptor activation. Specifically, what is the appropriate amount of GLP1R activation that buffer the hyperglycemic risk posed by GCGR activation. When a long-acting dual agonist was given to *Glp1r*$^{-/-}$ mice, the decrease in body weight was no longer associated with improvement in glucose metabolism (Day *et al*. 2009), highlighting the importance of an appropriate GLP1R engagement in preventing GCGR-mediated increase in glucose production. A recent report using a spectrum of receptor selectivity demonstrated that a dual agonist peptide with comparable functional potencies at the GLP1R and GCGR maximizes the weight loss and minimizes the hyperglycemic risk associated with GCGR activation in mice (Day *et al*. 2012). Another OXM analog, OXM6421, when injected in lean mice was observed to have a longer half-life than endogenous OXM and resulted in reduced intake of food as well as enhanced expenditure of energy (Liu *et al*. 2010). ZP2929, a chimeric peptide capable of fully activating both GLP1R and GCGR, improved glycemic control without body weight gain in db/db mice when combined with long-acting insulin (Fosgerau *et al*. 2011). Recently, Zealand Pharma announced the starting of a Phase I development of ZP2929 for the treatment of T2DM and/or obesity (Diabetes.co.uk, Sep 14, 2012) and Transition Therapeutics confirmed in a press release the completion of a Phase 1 study with TT-401, a weekly GLP1R/GCGR dual agonist developed for the treatment of diabetes (www.transitiontherapeutics.com/media/news.php, June 6, 2012).

Conclusion

Several trials have clearly demonstrated that lifestyle interventions (UKPDS 1995) and T2DM therapies such as sulfonylureas, metformin, and TZD result in progressive deterioration of glycemic control in T2DM patients associated with β-cell decline (Kahn *et al*. 2006). Therefore, restoration of insulin secretion and β-cell mass and function is a critical goal of future diabetes treatments. Thus far, incretin-based therapies (GLP1R agonists and DPP4 inhibitors) are providing durable glycemic control with improved insulin resistance and β-cell function (Klonoff *et al*. 2008, Derosa & Maffioli 2012, Derosa *et al*. 2012*a*,*b*, van Genugten *et al*. 2012).

With these initial promising results from incretin mimetics and incretin enhancers, the next generation of diabetes drugs will likely focus on the alternate delivery for injectables (Owens 2002, Lee *et al*. 2009, Sloop *et al*. 2010) and the combined activation of more than one receptor. Simultaneous activation of GLP1R and GCGR with chimeric peptides and in the future nonpeptide orally available GLP1R/GCGR dual agonists is a conceivable option to achieve improved therapeutic goals. It will be critical to deepen our understanding of the mechanism of action and how structurally related peptides like GLP1 and glucagon interact with their respective receptors. Understanding of receptor oligomerization, heteromerization, and binding cooperativity will allow an improved understanding of how ligands should be designed to maximize the simultaneous activation of these complexes (Roed *et al*. 2012, Schelshorn *et al*. 2012, Whitaker *et al*. 2012). Finally, characterization of the post-receptor signaling of these closely related GPCR in the glucagon family will allow a better understanding of the pathways that need to be selectively modulated to achieve the desired effect while avoiding others responsible for undesirable adverse effects.

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