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REVIEW

GLP-1R and amylin agonism in metabolic disease: complementary mechanisms and future opportunities

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The discoveries of the incretin hormone glucagon-like peptide-1 (GLP-1) and the β-cell hormone amylin have translated into hormone-based therapies for diabetes. Both classes of molecules also exhibit weight-lowering effects and have been investigated for their anti-obesity potential. In the present review, we explore the mechanisms underlying the physiological and pharmacological actions of GLP-1 and amylin agonism. Despite their similarities (e.g. both molecular classes slow gastric emptying, decrease glucagon and inhibit food intake), there are important distinctions between the central and/or peripheral pathways that mediate their effects on glycaemia and energy balance. We suggest that understanding the similarities and differences between these molecules holds important implications for the development of novel, combination-based therapies, which are increasingly the norm for diabetes/metabolic disease. Finally, the future of GLP-1- and amylin agonist-based therapeutics is discussed.

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Abbreviations
AmyKO, amylin knockout; AP, area postrema; ARC, arcuate nucleus of the hypothalamus; DIO, diet induced obesity; DPP-4, dipeptidyl peptidase-4; GLP-1, glucagon-like peptide-1; GLP-1R, glucagon-like peptide 1 receptor; GLP-2, glucagon-like peptide-2; NTS, nucleus of the solitary tract; OVX, ovariectomized; PVN, paraventricular nucleus of the hypothalamus; PYY(3–36), peptide YY(3–36); RAMP, receptor activity modifying protein; RSM, response surface methodology; SFO, subfornical organ

Introduction

Worldwide estimates suggest that over 1 billion individuals are overweight and 300 million are obese. Obesity and excess weight are established risk factors for diseases like type 2 diabetes mellitus. For obesity, available pharmacological therapies are lacking, with more obesity agents being withdrawn from the market over the past decade relative to those actually approved. The last two decades have seen a dramatic increase in our understanding of the neurohormonal controls of energy balance and a variety of neurohormones have been explored for their anti-obesity potential as mono- and combination-therapies (Morton *et al.*, 2006; Field *et al.*, 2009; Cegla *et al.*, 2010). Like obesity, diabetes mellitus is a multihormonal disease that afflicted 171 million people worldwide in 2010 and prevalence is projected to rise to 366 million by the year 2030 (World Health Organization, 2011). To maintain normoglycaemia, a complex, well-orchestrated interplay among several biological processes is required. Insulin secretion and action, β-cell health, glucagon secretion, energy intake and gastric emptying all play a significant role in determining glucose concentrations in the circulation. Relative to obesity, the available arsenal for the treatment of diabetes is larger with traditional approaches addressing insulin insufficiency through dietary/lifestyle modification and/or overcoming insulin resistance with oral agents that improve glycaemia through mechanisms that include reduction in hepatic glucose production or increases in the sensitivity and/or release of insulin (e.g. metformin, thiazolidinediones, sulfonylureas). However, some of these

therapies are associated with weight gain and cardiovascular risk, and ultimately do not address the progressive decline of the β-cell in diabetes. When these modalities fail individuals ultimately require exogenous insulin therapy.

These traditional views regarding the physiological and pharmacological regulation of glycaemia significantly evolved following the discoveries of the incretin hormone glucagon-like peptide-1 (GLP-1) and the β-cell hormone amylin in the mid-1980s. Both have translated into hormone-based therapies for diabetes in the forms of GLP-1 receptor (GLP-1R) agonists (e.g. exenatide) and the amylin agonist, pramlintide. Both classes of therapeutics also exhibit weight-lowering effects and have been investigated for their anti-obesity potential. On the surface, their glucose- and weight-lowering effects are orchestrated through seemingly overlapping physiologies. For example, although insulinotropism is unique to GLP-1 agonism, both agents decrease food intake, slow gastric emptying and decrease postprandial glucagon excursions. However, a careful examination of the receptor distribution, pharmacology, peripheral physiology and neurobiology of GLP-1- versus amylin-ergic systems reveals important differences between how these agents coordinate their physiological effects. These differences have significant implications for the development of novel, combination-based therapies, which is increasingly the norm for diabetes/metabolic disease. Hence, the focus of this review is to detail the mechanisms underlying the physiological and pharmacological actions of GLP-1 and amylin agonism and speculate on what future therapeutic modalities invoking these systems may hold.

Basic physiology

GLP-1

Glucagon-like peptide-1 is a product of the preproglucagon gene (Lund *et al.*, 1982), produced primarily by the L-cells of the distal intestine, although immunoreactive GLP-1 cells have also been localized in the mid-small intestine (Mortensen *et al.*, 2003). GLP-1 secretion is stimulated by nutrient ingestion and the peptide is present in multiple forms (1-36, 1-37, 7-36, 7-37). The bioactivity of the forms and degradation products of GLP-1 is an ever-changing landscape, with recent reports documenting important effects of a new cleavage product GLP(28–36) targeting mitochondria and modulating oxidative phosphorylation (Tomas *et al.*, 2011). Nevertheless, the most well-researched and important biologically active cleavage product appears to be GLP-1(7–36) amide. GLP-1's physiological effects include increasing glucose-stimulated insulin secretion, suppressing glucagon, controlling the rate of gastric emptying and reducing food intake (Holst, 2007). Following secretion it is rapidly inactivated (half-life ~2 min in rats) by dipeptidyl peptidase-4 (DPP-4) (Mentlein *et al.*, 1993). To overcome the pharmacological limitations of GLP-1's rapid degradation, drugs that are resistant to DPP-4 degradation (incretin mimetics or GLP-1R agonists) or which increase endogenous GLP-1 concentrations towards the normal range (DPP-4 inhibitors) have been developed.

Amylin

Amylin is a 37 amino acid peptide secreted from the pancreatic β-cells (Ogawa *et al.*, 1990). It is co-secreted with insulin in response to nutrient intake and the secretion of amylin is anatomically linked to insulin secretion with the diurnal patterns of amylin and insulin plasma concentrations being almost super-imposable (Koda *et al.*, 1992; Weyer *et al.*, 2001). Approximately 60% of immunoreactively detected amylin is glycosylated, although these forms were inactive in *in vitro* assays and their biological relevance is not well understood (Young, 2005b). Nephrectomy studies suggest that amylin is primarily cleared renally but also catabolized by renal peptidases associated with the vascular supply (Vine *et al.*, 1998). Pharmacokinetic studies that the terminal half-life of amylin in rats is ~13 min and that the half-life for the amylin agonist pramlintide in humans is ~20–45 min (Colburn *et al.*, 1996; Young, 2005a). Amylin's gluco-regulatory actions complement those of insulin by regulating the rate of appearance of glucose in the circulation and are achieved through three primary mechanisms: slowing the rate of gastric emptying, suppression of post-meal glucagon secretion and suppression of food intake. The clinical utility of human amylin is limited by instability and a propensity for self-aggregation. These challenges were overcome by the substitution of prolines at positions 25, 28 and 29 of human amylin (Colburn *et al.*, 1996). This resulted in a synthetic amylinomimetic peptide, pramlintide, with improved stability and decreased potential for aggregation.

Receptor pharmacology

GLP-1 receptors

The GLP-1R was originally cloned from a rat pancreatic islet cDNA library in 1992 (Thorens, 1992). The human receptor was isolated by several groups in 1993 and found to be 95% homologous to the rat protein (Dillon *et al.*, 1993; Graziano *et al.*, 1993; Thorens *et al.*, 1993). The GLP-1R is a member of the class B GPCRs (secretin family) and is 463 amino acids in length. Briefly, Class B receptors are classic seven transmembrane proteins whose C-terminus interacts with a signalling G protein and whose large N-terminal extracellular domain plays an important role in ligand binding (Parthier *et al.*, 2009). GLP-1R signalling mainly occurs through a Gαs coupled subunit that leads to increased cAMP (cyclic adenosine monophosphate) and Ca^{2+} concentrations. However, these second messengers can also be increased via signalling through other G protein subunits such as G αq, G αo and Gαi, activating other downstream signalling pathways [e.g. protein kinase A, protein kinase C, Phosphatidylinositol 3-kinase, exchange proteins activated by cAMP and MAPK pathways (Doyle and Egan, 2007)]. Subsequent expression studies have detected receptor transcripts in multiple tissues and in various species. In mice, mRNA was found in pancreatic islets, brain, heart, kidney and the GI tract (Campos *et al.*, 1994). In rats, a similar but expanded profile of mRNA expression identified transcript in pancreatic islets, lung, hypothalamus, stomach, heart and kidney (Bullock *et al.*, 1996). *In situ* hybridization studies have localized GLP-1R's in regions of the brain classically implicated in regulating energy homeostasis (e.g.

brainstem and hypothalamus) as well as other regions (Merchenthaler *et al.*, 1999). More recently, a role for lingual GLP-1 receptors has been proposed (Shin *et al.*, 2008). Notable species differences in receptor expression include the observation that rats have higher expression in lung tissue and thyroid C cells than humans (Korner *et al.*, 2007; Bjerre Knudsen *et al.*, 2010). Although dogs have detectable GLP-1R expression in muscle and adipose tissue (Sandhu *et al.*, 1999), there is controversy about whether or what form of GLP-1 receptors are present in human and rodent muscle, adipose tissue and liver in view of GLP-1 activity on those tissues (Campos *et al.*, 1994; Egan *et al.*, 1994). The biological relevance of all of these inter-species comparisons will also depend upon a host of other factors including the prevailing endogenous hormone concentrations and receptor affinities within each species.

Amylin receptors

As with GLP-1, amylin stimulates receptors containing a class B GPCR. However, with the amylin receptor there is an added layer of complexity due to the fact that the base GPCR heterodimerizes with a receptor activity modifying protein (RAMP). RAMPs are a family of three type I transmembrane proteins composed of a large N-terminal extracellular domain, a single alpha helical transmembrane domain and a short intracellular section. These proteins regulate receptor pharmacology, receptor signalling and receptor trafficking [originally described in McLatchie *et al.* (1998)] and for a recent review see Sexton *et al.* 2009). RAMPs were initially shown to regulate and modify the calcitonin receptor-like receptor to form distinct receptors for calcitonin gene-related peptide and adrenomedullin. Subsequent work found that RAMPs also modify the pharmacology of the calcitonin receptor such that specific amylin receptors emerge from the dimerization of various splice variants of the calcitonin receptor (CT(a)/CT(b)) with either RAMP1, RAMP2 and RAMP3. These receptors are commonly referred to as AMY_1, AMY_2 and AMY_3 with either an a or b in the subscript to define which splice variant of the calcitonin receptor is in the complex (Poyner *et al.*, 2002). Overall, the distribution of amylin receptors appears to be more localized relative to GLP-1R. Amylin receptors have been localized via binding studies to the nucleus accumbens, the dorsal raphe and the area postrema (AP) in the hind brain (Beaumont *et al.*, 1993). *In situ* studies in rats revealed that $AMY_{2(a)}$ and $AMY_{3(a)}$ are the amylin receptor subtypes localized to the AP suggesting these subtypes are responsible for the satiogenic effect of amylin (Barth *et al.*, 2004) (discussed in further detail below). Within the AP, the key second messenger system appears to be cGMP (Riediger *et al.*, 2001). Messenger RNA for calcitonin receptor-like receptor + RAMP1 or RAMP2 are found in the subfornical organ (SFO) and are likely responsible for amylin's induction on drinking (Barth *et al.*, 2004). RT-PCR studies demonstrate that RAMP1 and 2 but not RAMP3 are present in the rat nucleus accumbens suggesting that the amylin receptor in the nucleus accumbens is either AMY_1 or AMY_2 (Li *et al.*, 2004). The role of these amylin receptors, as well as their cognate ligand, has not been well established but has been proposed to potentially link amylin's regulation of motor activity and ingestive behaviour (Baldo and Kelley, 2001). Infusion studies suggest that amylin crosses the blood–brain barrier potentially accessing a number of regions (e.g. cerebellum, midbrain, frontal cortex, parietal cortex and occipital cortex) (Banks *et al.*, 1995; Banks and Kastin, 1998), although the physiological relevance of these findings merits further investigation.

Overlap of CNS GLP-1 and amylin receptors and signalling

Amylin-activated cells (by c-Fos (Potes *et al.*, 2010) as well as GLP-1R-positive cells (Yamamoto *et al.*, 2003) have been localized to tyrosine hydroxylase containing cells within the hindbrain AP, although double labelling of amylin and GLP-1R's has never been performed. Both amylin and GLP-1 appear to activate somewhat overlapping neural circuits. GLP-1 agonism induces c-Fos in the AP, caudal nucleus of the solitary tract (cNTS), the lateral parabrachial nucleus, hypothalamic paraventricular nucleus (PVN), bed nucleus of the stria terminalis and central nucleus of the amygdala (Rowland *et al.*, 1997; Baggio *et al.*, 2004). Likewise, amylin induces c-Fos expression in the AP, NTS, lateral parabrachial nucleus and the central nucleus of the amygdala. The AP is far more important for transducing the effects of amylin relative to GLP-1. AP lesions abolish the anorexigenic effects of amylin (Lutz *et al.*, 1998b; Rowland and Richmond, 1999; Riediger *et al.*, 2004; Becskei *et al.*, 2007; Mack *et al.*, 2010), whereas those of GLP-1 are maintained (Baraboi *et al.*, 2010). Studies using [Ser8]GLP-1 (a more stable analogue of GLP-1) suggest that peripheral GLP-1 can cross the blood–brain barrier by simple diffusion (Kastin *et al.*, 2002). However, because of its rapid degradation by DPP-IV, to what extent under physiological conditions peripheral GLP-1 actually reaches the CNS has been debated. Gut-derived GLP-1's central effects are most likely propagated via interactions with afferent vagal nerve fibres arising from the nodose ganglion, sending impulses to the NTS and onwards to the hypothalamus (Vahl *et al.*, 2007). The NTS does contain a population of GLP-1 synthesizing neurons that project to the PVN (Larsen *et al.*, 1997), representing another potential point of overlap between amylin and GLP-1 signalling. Irrespective of their primary mechanisms for activating CNS neural circuits, both peptides have been shown to regulate the activity of a number of key neurohormonal signals (e.g. neuropeptide Y, pro-opiomelanocortin, leptin, cholecystokinin) in a variety of nuclei (e.g. AP, NTS, ARC, PVN, ventromedial hypothalamus) implicated in energy homeostasis (Roth *et al.*, 2006; Seo *et al.*, 2008; Becskei *et al.*, 2009; Skibicka *et al.*, 2009; Williams *et al.*, 2009; Turek *et al.*, 2010).

Central and peripheral signalling pathways

In this section we compare and contrast (when known), the primary pathways mediating the effects of GLP-1 and amylin agonism to suppress glucagon, slow gastric emptying and decrease food intake. These effects are summarized in Table 1.

Insulinotropic and direct β-cell effects

Early *in vitro* studies demonstrate that GLP-1 can act directly upon pancreatic β-cells to stimulate insulin secretion. These

Table 1

Summary of Features and Mechanisms Reviewed

	GLP-1	Amylin
Source	• Intestinal L-cells • Neurons in NTS	• Pancreatic β-cells
Receptor and distribution	• Class B GPCR • Widespread receptor distribution (e.g. islets, brain, heart, kidney, gastrointestinal tract)	• Class B GPCR + RAMP • Localized CNS distribution – key receptors in hindbrain AP. Also expressed in nucleus accumbens, dorsal raphe and SFO
β-cell	• Insulinotropic/Amylinotropic • Increased β-cell mass via direct and indirect mechanisms	• Co-secreted and co-released with insulin
Glucagon secretion	• Glucagonostatic (with 'hypoglycaemic' override) • Direct and indirect/paracrine mechanisms reported	• Glucagonostatic (with 'hypoglycaemic' override) • Activation of AP and then vagally transmitted (proposed)
Gastric emptying	• Decreases rate • Current evidence favours neural reflex triggered by peripheral GLP-1 activating vagal afferents innervating the gut/portal area	• Decreases rate • Activation of AP, then transmitted by vagal efferents
Food intake	• Decreases intake • Central and peripheral mechanisms	• Decreases intake • Mediated via direct effects on AP • Intact vagus not required
Available knockout models	• Proglucagon peptides ($Gcg^{gfp/gfp}$) • $GLP\text{-}1R^{-/-}$ • Double incretin receptor knockout ($GLP\text{-}1R^{-/-}$ and $GIP\text{-}R^{-/-}$)	• Amylin deficient mice • No specific receptor knockouts • RAMPS (only) have been deleted or over-expressed • Calcitonin receptor knockouts
Future therapeutic approaches in metabolic disease	• Analogueing and improved delivery • Hormonal combinations • Allosteric modulators • 'Phybrids'/Chimeras	• Analogueing and improved delivery • Hormonal combinations (e.g. amylin/leptin synergy evident in preclinical and clinical studies) • 'Phybrids'/Chimeras
Other disease areas of note	• Neurodegenerative diseases (Parkinson's, Alzheimer's)	• Neuropsychiatric diseases (anxiety, depression)

mechanisms are likely invoked under pharmacological conditions with stable GLP-1R agonists; however, under normal physiological conditions whether endogenous GLP-1 from L-cells can access β-cells before degradation is often debated (Williams, 2009). Other intermediary mechanisms have been proposed. For example, the observation that hepatic portal GLP-1 increased activity of pancreatic vagal fibres (Nakabayashi *et al.*, 1996) led to a series of studies that suggest that β-cell stimulation by portal GLP-1 is evoked by a neural reflex (shown to be non-muscarinic) that may be triggered in the liver (Balkan and Li, 2000). Central GLP-1 systems also activate pancreas-projecting preganglionic vagal motoneurons (Wan *et al.*, 2007). Within the pancreas, the stimulatory effects involve multiple signal transduction events, including ion channel activity, intracellular Ca^{2+} handling, membrane docking and exocytosis of insulin-containing granules. In preclinical models, a number of reports also support the concept that GLP-1 agonism can increase β-cell mass via stimulation of β-cell neogenesis, stimulation of β-cell proliferation and suppression of β-cell apoptosis. These effects have all been extensively reviewed elsewhere (Gromada *et al.*, 2004; Nielsen *et al.*, 2004; Doyle and Egan, 2007). The effects of amylin have been explored in isolated β-cells, isolated pancreatic islets and perfused pancreas, and amylin has not been shown to possess insulinotropic properties. Amylin exerts insulinostatic effects *in vitro*, potentially mediated via an amylin-like receptor linked to Gi-mediated inhibition of cAMP in islets [for a historical/methodological perspective on the *in vivo* relevance of these studies see Young (2005d)].

Glucagon suppression

The glucagonostatic effects of both peptides are important from a therapeutic perspective as patients with diabetes have inappropriate elevations in fasting and postprandial glucagon. Although GLP-1 is clearly a potent inhibitor of glucagon, the direct mechanisms by which GLP-1 reduces glucagon secretion have not been well established. Whether GLP-1Rs are even expressed on α-cells is controversial. Receptors have been detected by some techniques, but not others, and surprisingly, GLP-1 application tends to increase gluca-

gon secretion in *ex vivo* preparations [isolated rat and mouse α-cells; reviewed in Gromada and Rorsman (2004)]. The action of GLP-1 on glucagon secretion has been proposed as a paracrine mechanism, mediated by stimulated release of somatostatin that in turn suppresses exocytosis in the α-cell (Ding *et al.*, 1997; de Heer *et al.*, 2008). The potent insulinotropic actions of GLP-1 have been suggested as contributing to direct insulin-mediated suppression of glucagon (Unger and Orci, 2010). Like GLP-1, amylin's glucagonostatic properties also do not appear to be achieved by a direct effect of amylin upon α-cells as evidenced by a lack of effect in *ex vivo* (e.g. isolated perfused pancreas) or *in vitro* [e.g. isolated pancreatic islets Silvestre *et al.* (2001)] preparations. Rather, amylin's effects are most likely initially mediated via activation of hindbrain receptors, which in turn activate the vagus nerve, although this remains to be demonstrated experimentally. It is important to note that for both hormones, under conditions when blood glucose is low (e.g. fasting), their actions to inhibit glucagon are not present representing an important protective effect against developing hypoglycaemia (Gedulin *et al.*, 1997; Nauck *et al.*, 2002). This phenomenon is referred to as a 'hypoglycaemic override mechanism' and is a desirable feature from a therapeutic perspective.

Gastric emptying

Several groups have explored the mechanisms mediating the gastric emptying effects of GLP-1. The role/s of peripheral versus central GLP-1 and vagal transmission of the gastric emptying effects has received much attention. In rats, vagotomy decreased intestinal secretion of GLP-1 (Rocca and Brubaker, 1999) as well as its gastro-inhibitory effects (Holmes *et al.*, 2009). A series of experiments characterized the actions of GLP-1 on gastric projecting dorsal motor nucleus of the vagus (Holmes *et al.*, 2009). Using electrophysiology, subpopulations of gastric-projecting dorsal motor nucleus of the vagus neurons were found to be depolarized by exogenously applied GLP-1 and exenatide. Microinjection of GLP-1 or exenatide to the brainstem decreased gastric tone, and selective vagotomy studies implicated ipsilaterally projecting motoneurons in mediating these gastric effects. These effects appear to be transmitted via activation of non-cholinergic non-adrenergic inhibitory pathways rather than by inhibiting tonic excitatory cholinergic pathways (Holmes *et al.*, 2009). As noted above, although intestinally secreted GLP-1 can cross the blood–brain barrier (Kastin *et al.*, 2002), given its rapid degradation, this scenario is unlikely. Under normal physiological conditions, the proposed source of GLP-1 is from proximal GLP-1 synthesizing neurons within the NTS acting in a neurotransmitter-like manner upon the dorsal vagal complex. At higher pharmacological levels, GLP-1 and/or its more stable analogues could engage both central and peripheral mechanisms. In pigs, the effect of GLP-1 on gastric motility does not appear to require an intact vagus nerve suggesting there may be species differences (Nagell *et al.*, 2006). In humans, clinical observations suggested the importance of an intact vagal pathway, as the actions of GLP-1 on other gastric functions such as decreasing gastric acid secretion were no longer evident in individuals that had undergone vagotomy due to duodenal ulcers (Wettergren *et al.*, 1997). Clinical studies have gauged the contribution of endogenous GLP-1 on gastric emptying using the GLP-1R antagonist exendin(9–39). Unfortunately, owing to the use of different populations and tests meals, results to date have been contradictory, with reports of gastric emptying either being accelerated, slowed or unchanged (Salehi *et al.*, 2008; Deane *et al.*, 2010; Nicolaus *et al.*, 2011; Witte *et al.*, 2011). In contrast to GLP-1, amylin inhibition of gastric empting is thought to be primarily centrally mediated. First, aspiration of the AP abolishes amylin's effects on gastric emptying (Young, 2005c). More recently, amylin inhibition of gastric emptying was demonstrated in rats with a sub-diaphragmatic vagal deafferentation (e.g. in which vagal afferent fibres are destroyed but efferent fibres remain intact) supporting the hypothesis that amylin acts via hormonal mechanisms to stimulate the AP and transmits signals to the gut via efferent vagal fibres (Wickbom *et al.*, 2008).

Food intake

The inhibitory effects of peripherally applied GLP-1 on food intake are attenuated in surgically vagotomized rats (Abbott *et al.*, 2005) and chemically (capsaicin)-treated mice (Talsania *et al.*, 2005). Careful pharmacological studies have compared peripheral routes of administration of GLP-1 on meal size in sham and vagotomized rats. Anorexigenic effects of intraperitoneal but not hepatic portal vein GLP-1 required intact vagal afferent signalling, but intravenous GLP-1 most likely acts centrally and not via activation of hepatic GLP-1 receptor systems (Ruttimann *et al.*, 2009). The effects of centrally administered GLP-1 were abolished by perinatal ARC lesions [using monosodium glutamate (Tang-Christensen *et al.*, 1998)]. Overall, CNS lesions have not yielded specific nuclei that are necessary for the expression of peripheral GLP-1's anorexigenic effects. For example, a recent series of studies investigated the role of two circumventricular organs, the AP and the SFO, both of which are known to possess GLP-1 binding sites (Orskov *et al.*, 1996; Yamamoto *et al.*, 2003), in mediating the metabolic and CNS-stimulating effects of GLP-1. Neither electrolytic lesion of the AP, the SFO or combined AP + SFO meaningfully altered the anorexigenic effects of peripherally applied exenatide, a GLP-1R agonist (Baraboi *et al.*, 2010). To what extent GLP-1 or its receptor agonists need to cross the blood–brain barrier to exert any of their physiological effects is debated. For example, intraperitoneal injections of albumin-bound GLP-1 molecules, which are of sufficient size to be excluded from the blood–brain barrier, still decrease food intake and delay gastric empting and activate a similar pattern of neuronal activity as exenatide (Baggio *et al.*, 2004). These findings point to a model whereby peripheral activation of GLP-1R-dependent vagal afferents is capable of activating CNS centres and transducing the effects of GLP-1 in the brain. Nevertheless, there may be important differences when considering pharmacological effects of agonism using a GLP-1 'backbone' versus exenatide. For example, in contrast to GLP-1, the central but not peripheral anorexigenic properties of exenatide were shown to be insensitive to GLP-1R antagonism suggesting that there may be important differences between these ligands (Barrera *et al.*, 2009). CNS GLP-1 systems have been implicated in mediating non-homeostatic influences on ingestive behaviour, most notably malaise (Thiele *et al.*, 1997). GLP-1R agonism increases taste aversion learning and pica behaviour, and GLP-1R antagonism inhibits the formation of taste aversion

in response to known emetic agents such as lithium chloride (Seeley *et al.*, 2000). Separate populations of GLP-1 receptors may mediate the anorexigenic versus aversive effects of GLP-1 agonism (Kinzig *et al.*, 2002) as intra-amygdala injections of GLP-1 produce taste aversion learning but not anorexigenic effects whereas brainstem injections of GLP-1 reduce food intake but do not condition aversions. To what extent the satiogenic and malaise effects can be dissociated is frequently debated (Tang-Christensen and Cowley, 2007). Clinical experience with GLP-1 agonism suggests that there is nausea that occurs during the early days of treatment and, in most individuals, decreases with time and/or gradual dose escalation.

The neural circuitry required for the expression of amylin's anorexigenic effects is well understood. As noted above, an intact AP is necessary for the expression of amylin's anorexigenic effects. Direct AP application of low doses of amylin inhibits food intake, whereas antagonism of AP receptors (with AC187) increases food intake and abrogates the anorexigenic effects of exogenous peripheral amylin (Mollet *et al.*, 2004). Rats with a specific hepatic branch vagotomy (Lutz *et al.*, 1995), total sub-diaphragmatic vagotomy (Lutz *et al.*, 1994) or capsaicin-induced lesions of peripheral neural afferents that project to the brain (Lutz *et al.*, 1998a) still respond to amylin implying that an intact vagus is not required for amylin's anorexigenic effects. Under normal physiological conditions vagal transmission and gastric emptying likely serve to enhance amylin's central effects. For example, amylin suppression of 'sham' feeding (e.g. a technique where liquid food drains from the stomach so that gastric food stimuli related to the accumulation of food in the stomach, intestinal food stimuli and post-absorptive food stimuli are absent or greatly reduced) requires higher doses compared with those required to decrease 'real' feeding (Asarian *et al.*, 1998). An interesting preclinical difference between amylin versus GLP-1 agonism is noted in preclinical assays of malaise. Amylin is not associated with malaise (taste aversion or pica) or competing locomotor behaviours (e.g. hyperactivity or immobility would prevent food consumption) in rats or mice (Chance *et al.*, 1992; Morley *et al.*, 1997; Rushing *et al.*, 2002; Mack *et al.*, 2007). Nausea is the most common tolerability-related adverse event with pramlintide treatment; however, it is generally mild and transient and is dissociable from weight loss (Aronne *et al.*, 2007). In a clinical study approximately 76% of patients who received pramlintide achieved a significant reduction in body weight but did not report any malaise (Hollander *et al.*, 2004). This is another example, wherein amylin and GLP-1 seem to strongly activate similar brain regions and circuits (e.g. the AP a region known to be important for emesis), with different whole organism effects. Another interesting feature with amylin agonism relates to dietary selection. When amylin-treated diet induced obesity (DIO) rats were allowed to self-selecting calories from either a low- or high-fat diet, they consistently chose a larger percentage of calories from the low-fat diet relative to controls (Mack *et al.*, 2007). In clinical studies, pramlintide has also been shown to reduce acute and sustained reductions in the intake of highly palatable high-fat, high-sugar foods at a 'fast-food challenge' (Smith *et al.*, 2007) suggesting that some of these nuances of feeding behaviour observed in preclinical models may be recapitulated in the clinic. To what extent these characteristics are unique to amylin agonism warrants further investigation.

Insights from knockout models

GLP-1 receptor knockout

Proglucagon encodes multiple bioactive products (e.g. glucagon, GLP-1, GLP-2) precluding the generation of a GLP-1 specific knockout. Knock-in mice deficient in all peptides were generated by introduction of a green fluorescent protein cDNA ($Gcg^{gfp/gfp}$). Overall their phenotype was fairly mild with α-cell hyperplasia noted, somewhat lowered blood glucose concentrations and slight increases in body weight (Hayashi *et al.*, 2009). Mice with a targeted disruption of the GLP-1R gene have also been created ($GLP\text{-}1R^{-/-}$ (Scrocchi *et al.*, 1996) and reviewed in (Hansotia and Drucker, 2005). Islets isolated from these mice exhibit abnormal basal and glucose-stimulated cytosolic Ca^{+} accumulation (Flamez *et al.*, 1999). Histological irregularities were noted in islet cell size and number as well as in the distribution of α-cells (Ling *et al.*, 2001). The phenotype of $GLP\text{-}1R^{-/-}$ mice includes modest gluco-regulatory perturbations such as mild fasting hyperglycaemia, glucose intolerance in response to oral and intraperitoneal glucose tolerance tests and diminished glucose-stimulated insulin secretion. Given the glucagonostatic effects of GLP-1 it was surprising that fasting and postprandial glucagon concentrations were normal in the knockouts. With respect to energy balance, $GLP\text{-}1R^{-/-}$ mice maintain normal body weight and food intake, even in aged mice maintained on a high-fat diet (Scrocchi and Drucker, 1998). Intriguingly, $GLP\text{-}1R^{-/-}$ mice are protected from high-fat diet induced muscle and hepatic insulin resistance (Ayala *et al.*, 2010). Central or peripheral leptin responsiveness of $GLP\text{-}1R^{-/-}$ mice was also explored and shown to be similar to that in WT controls (Scrocchi *et al.*, 1997). Because the GLP-1R knockout phenotype was so modest, it was speculated that endogenous gastric inhibitory polypeptide (GIP; the other incretin) was compensating for these effects and double GLP-1/GIP receptor knockouts were created. Although oral glucose tolerance was somewhat worsened in GLP-1/GIP double knockout mice further perturbations in food intake, body weight, insulin-induced hypoglycaemia and glucagon effects were not noted (Hansotia *et al.*, 2004).

Amylin knockout (AmyKO)

With respect to amylin, the complexity of multiple RAMPs and calcitonin receptors makes the exploration of specific receptor knockouts difficult. Global deletion of the calcitonin receptor has revealed physiological roles for the receptor in bone biology (e.g. to protect against hypercalcaemia), but no effects on body weight or metabolic disease-related endpoints were noted (Davey *et al.*, 2008; Turner *et al.*, 2011). Individual RAMPs have been disrupted revealing a range of phenotypes. Mice with a disrupted RAMP1 gene were hypertensive and exhibited a dysregulated immune response, removal of RAMP2 was lethal and RAMP3 knockout mice appear normal until old age when they were not as heavy as their wild-type littermates (Sexton *et al.*, 2009). An interesting recent report demonstrated that mice with over-expression of RAMP1 had

increased energy expenditure as well as enhanced responsiveness to amylin (Zhang *et al.*, 2011). The role of endogenous amylin, however, was originally investigated in peptide deficient (AmyKO) mice originally created on a 129Ola/B6 background (Gebre-Medhin *et al.*, 1998). Basal insulin and glucose concentrations were unchanged in AmyKO mice relative to wild types throughout development. If anything, the knockouts demonstrated slight gender-specific effects to increase insulin secretion and glucose tolerance. The lack of endogenous amylin was noted to worsen alloxan-induced diabetes pointing to a potential benefit in situations of β-cell damage (Mulder *et al.*, 2000). In terms of weight regulation, modest, transient elevations in weight gain were noted especially in AmyKO males maintained on standard low fat diet (Gebre-Medhin *et al.*, 1998). To further gauge the impact of amylin insufficiency on energy balance the mixed background AmyKO mice were backcrossed onto the more commonly used C57/B6 background, which readily develops high-fat diet-induced obesity (Turek *et al.*, 2010). With the exception of a temporary increase in adiposity in AmyKO females, no differences in body weight, body composition or plasma leptin concentrations were observed when mice were maintained on a low-fat diet (6 weeks). AmyKO mice did not have an increased propensity to develop obesity after being switched to a high-fat diet (27 weeks). However, by contrast to GLP-1R$^{-/-}$ mice, marked differences were noted in response to exogenous leptin challenges (Turek *et al.*, 2010). Lean AmyKO exhibited diminished central leptin signalling in response to peripheral leptin injections. Leptin-induced phosphorylated signal transducer and activator of transcription levels in the hypothalamus and hindbrain only achieved 60% the levels of stimulation observed in wild-type controls. While initially effective, the weight-reducing effects of exogenous leptin were ultimately less durable (in female AmyKO) or completely absent (in male AmyKO) over the course of a 4 week peripheral infusion. AmyKO mice also had a 50% reduction in mRNA for the long form of the leptin receptor (Turek *et al.*, 2010), and amylin has been recently proposed to play a role as a trophic factor (Potes and Lutz, 2010).

The relatively modest phenotypes observed in the GLP-1R$^{-/-}$ and AmyKO models may also be attributable to the reliance on germ line knockouts in which multiple compensatory mechanisms may be engaged during development. For a variety of reasons (e.g. difficulty in measuring food intake, frequency of meal-taking and responses to food deprivation) mice may also not represent the optimal species for discerning the role/s of these signals in energy homeostasis. The importance of amylin and GLP-1 in normal physiology and their relevance as therapeutic classes warrants the development of inducible (and potentially tissue-specific) knockouts and/or developing knockouts in other species (e.g. rats).

Human imaging studies

Complementary to the immunohistochemical neurobiological assessments of CNS pathways in rodents clarifying differences between GLP-1R and amylin agonism is the emerging application of neuroimaging data in humans. The effects of i.v. GLP-1(7–36) infusion on cerebral glucose metabolism were evaluated using positron emission tomography. Peripheral administration reduced carbohydrate metabolism within discrete regions of the brain, most notably in the hypothalamus and brainstem (Alvarez *et al.*, 2005). To assess whether postprandial changes in GLP-1 modulate neuronal activity, another study performed a *post hoc* correlation analysis of changes in $H_2{}^{15}O$-positron emission tomography measurements of regional cerebral blood flow relative to changes in plasma concentrations of GLP-1 after a meal (Pannacciulli *et al.*, 2007). The peak postprandial increases in plasma GLP-1 concentrations were associated with increases in rCBF in the left dorsolateral prefrontal cortex and the hypothalamus, both before and after adjustment for sex, age, body fat, and changes in plasma glucose, insulin, and serum free fatty acid concentrations. Although there are clear methodological and interpretational caveats associated with both techniques and study design (spatial resolution, contrast resolution of individual subtraction images, covariate analyses, etc.), the observation that the former study was associated with changes in hindbrain but not cortical regions whereas the latter implicated hypothalamic and cortical regions but not hindbrain points to regions that may be activated by pharmacological versus endogenous (meal-stimulated) GLP-1. In response to food cues (image viewing), individuals with type 2 diabetes mellitus have also been shown to exhibit enhanced activation of dopaminergic reward and motivation centres without activation of cholinergic self-awareness centres. A recent report suggests that exenatide can shift these responses to be more akin to that observed in non-diabetic individuals (Nathan *et al.*, 2010).

To our knowledge, although comparable data do not yet exist for amylin agonism as a monotherapy, a functional magnetic resonance imaging study recently compared activation following the administration of metreleptin with the combination of metreleptin + pramlintide. In this study, pramlintide significantly potentiated leptin activation in mesolimbic brain regions such as the hippocampus and amygdala (Klopfenstein *et al.*, 2010). To what extent these findings explain the established weight loss synergy between amylin and leptin agonism [discussed below (Roth *et al.*, 2008b; Trevaskis *et al.*, 2008)] remains to be determined. Functional neuroimaging in humans is clearly an area that warrants further research. Not only do these kinds of studies provide rich translational insights into the neurobiological substrates activated by these neuropeptides in humans but they may also serve as an important tool in identifying key pathways to target with combination-based therapeutics.

GLP-1/amylin agonism and additivity/synergy

The therapeutic potential of neurohormonal combinations has garnered significant recent attention. In this section we briefly review additive and synergistic interactions that have been described between other neurohormonal signals with GLP-1 or amylin, as well as interactions between GLP-1 and amylin.

Neurohormonal interactions with GLP-1 agonism

The marked anti-diabetic and weight-lowering effects of bariatric Roux-en-Y surgery have been associated with elevations in endogenous peptide YY(3–36) [PYY(3–36)] and GLP-1. As such, the combined effects of these hormones or their agonists have been studied by several groups. In fasted lean mice, single-dose combination studies revealed that co-injection of PYY(3–36) with exenatide exerted greater and more durable anorexigenic and gastric emptying effects relative to either agent alone over an 8 h period (Talsania *et al.*, 2005). Short-term additive anorexigenic effects have been reported with this combination in lean and genetically obese mice, as well as in a clinical study in lean, fasted volunteers (Neary *et al.*, 2005). However, the anti-obesity potential of this combination remains to be demonstrated as repeated injection of the combination failed to suppress weight gain in high-fat fed mice (Talsania *et al.*, 2005). Chronic intermittent administration of exenatide and PYY(3–36) at doses likely to reproduce plasma concentrations of GLP-1 and PYY(3–36) observed in Roux-En-Y gastric bypass patients also failed to produce a more prolonged reduction in daily food intake and body weight in DIO rats (Reidelberger *et al.*, 2011). Several groups have explored interactions between GLP-1 and leptin with mixed results. In lean rats, leptin pretreatment (at a low dose that had no effects when given alone) substantially increased the anorexigenic effects of GLP-1 or exenatide (Williams *et al.*, 2006). Counter to these behavioural effects was the surprising observation that leptin pretreatment blocked exenatide-induced c-Fos activation in several hindbrain regions pointing to the need for further mechanistic investigation/s. Another group tested multiple dose combinations of both agents in lean rats and observed transient additive anorexigenic effects noted only when exenatide was combined with a low dose of leptin to suppress 24 h food intake. Upon repeated administration (5 days) no significant effects beyond monotherapy were noted in lean rats (Bojanowska and Nowak, 2007). In our lab, in a single dose combination study in DIO leptin resistant rats, the exenatide analogue AC3174 exerted less than additive anorexigenic and weight lowering effects when combined with leptin (Roth *et al.*, 2008b). As discussed below, the same dose of leptin was shown to markedly and repeatedly synergize with amylin. Two short-term clinical studies have compared the individual and combined effects of GLP-1 and cholecystokinin infusion in lean healthy individuals and noted less than additive interactions to suppress acute food intake (Gutzwiller *et al.*, 2004; Brennan *et al.*, 2005).

Neurohormonal interactions with amylin agonism

Multiple combination studies have been conducted with amylin and other hormones including CCK, PYY(3–36) and leptin. Several of these studies used rigorous tests for synergy such as isobolographic analysis or response surface methodology (RSM) (Roth *et al.*, 2010). Using isobolographic analysis amylin was shown to synergistically suppress acute food intake in lean mice when combined with cholecystokinin (Bhavsar *et al.*, 1998). With sustained administration, durable anorexigenic and weight-lowering effects were demonstrated in leptin resistant DIO rats (Trevaskis *et al.*, 2010b). Neurobiology studies implicated cooperative activation of the AP particularly in noradrenergic neurons (Trevaskis *et al.*, 2010b). Amylin and PYY(3–36) exerted complementary effects to suppress food intake for up to 24 h in high-fat fed rats whereas the effects of the individual agents were no longer evident after 5 h (Roth *et al.*, 2007). Likewise, in DIO mice a single dose combination suggested mathematical additivity. An RSM study in DIO rats revealed that this combination inhibited food intake synergistically and reduced body weight additively (Roth *et al.*, 2007). As with amylin/cholecystokinin, co-activation of AP neurons may be an important feature of amylin/PYY(3–36) additivity (Potes and Lutz, 2010). Finally, a clinically relevant and reproducible synergy has been noted with combined amylin and leptin agonism. Central administration of leptin was shown to increase the acute eating-inhibitory effect of peripheral amylin in lean rats (Osto *et al.*, 2007). In co-administration studies we found that doses of peripherally administered leptin that had no appreciable effect on body weight in DIO leptin resistant rats enhanced amylin-mediated weight loss. An RSM study confirmed that the combination synergistically decreased both food intake and body weight (Trevaskis *et al.*, 2008). Although the combination of leptin with other anorexigenic peptides such as PYY(3–36) and a GLP-1 analogue also resulted in significant weight loss, neither combination induced the same extent of fat-specific weight loss as the combination of amylin and leptin. Several mechanisms of amylin and leptin synergy have been elucidated (Roth *et al.*, 2008b; Trevaskis *et al.*, 2008; Turek *et al.*, 2010) and recently reviewed (Trevaskis *et al.*, 2010a). In brief, amylin appears to restore and/or augments central leptin responsiveness in selected hypothalamic regions (most notably the ventromedial hypothalamus) by increasing leptin receptor number and signalling capacity. Another lab recently examined the cardiovascular effects of combined amylin and leptin treatment in lean and obese rats. In addition to decreasing body weight and adiposity the combination lowered blood pressure, and prevented bradycardia and metabolic suppression typically observed with negative energy balance (Seth *et al.*, 2010). The clinical significance and potential of amylin/leptin synergy was established in a translational clinical research study in which obese/overweight individuals treated with pramlintide/metreleptin induced significantly greater weight loss (12.7%) than treatment with pramlintide or metreleptin alone (Roth *et al.*, 2008b; Ravussin *et al.*, 2009).

Amylin-dependent actions of GLP-1?

As amylin and insulin are co-localized and co-secreted, GLP-1 can also be regarded as having 'amylinotropic' properties. To understand the contribution of endogenous amylin to the effects of GLP-1 recent elegant clinical investigations compared the effects of IV infusion of a low and high dose of GLP-1 (that achieved plasma concentration of ~100 pM or ~170 pM, respectively) in healthy individuals and individuals with type 1 diabetes mellitus. Because endogenous amylin is present in healthy individuals and absent in individuals with type 1 diabetes mellitus differences in GLP-1 modulation of gastric emptying, food intake and glucagon was attributed by the authors to the presence or absence of endogenous amylin

(Asmar *et al.*, 2010). In these studies, GLP-1 exerted similar effects on gastric emptying, food intake and appetite in both subject populations implying that GLP-1 can exert these effects in an amylin-independent manner. From a therapeutic perspective this supports the notion that these systems are not completely overlapping and points to the potential for combined GLP-1 and amylin agonism (discussed below).

GLP-1 and amylin combinations

Although both GLP-1 and amylin are released in response to a meal, and exert gluco- and food-intake regulatory effects, as discussed above, they do so through different mechanisms and sites of action. If GLP-1 and amylin pathways were truly overlapping (and redundant) then simultaneous agonism of both pathways should not achieve greater effects than maximal agonism of either pathway alone. On the other hand, if their pathways were complementary, it might be possible to achieve greater effects than that obtained by either pathway alone. Bello *et al.* (Bello *et al.*, 2010) tested this hypothesis in a nonhuman primate model by co-administering the amylin agonist salmon calcitonin alone and in combination with exenatide. Monkeys were maintained on a schedule of 6 h daily access to food and multiple dose combinations were used, which enabled the authors to use RSM to formally test for statistical additivity or synergy for suppression of food intake. The response surface indicated synergistic anorexigenic effects on hours 1–4 and additive effects during hours 5–6. Importantly, the monkeys did not display outward signs of malaise or nausea. Although the only dependent measure in this study was acute food intake, these non-human primate findings raise the possibility for additional gluco- and weight-regulation with combined GLP-1 and amylin agonism in the clinic.

Future therapeutic opportunities and considerations

Improved delivery

Glucagon-like peptide 1 receptor and amylin agonists are each currently dosed as QD/TID injection regimens. From a patient convenience perspective, this injection burden, while potentially adding efficacy, is clearly not attractive. The GLP-1 class is at a more advanced stage relative to amylin agonism and GLP-1 agents with weekly to monthly injection intervals at various stages of development (Baggio *et al.*, 2008; Malone *et al.*, 2009; Christensen and Knop, 2010; Madsbad *et al.*, 2011). These technologies rely either on formulation- (e.g. microspheres) or scaffold- (e.g. albumin binding or conjugation, Fc fusions or conjugates) based approaches to prolong the pharmacokinetic half-life of the peptide. Oral (Marino *et al.*, 2010; Steinert *et al.*, 2010) or inhaled (e.g. fumaryl diketopiperazine microspheres capable of carrying small peptides deep into the lungs (Marino *et al.*, 2010) preparations of GLP-1R agonists are being vigorously pursued but are still at very early stages.

Increase efficacy/potency

A classic drug development approach to improve upon these molecules would be to increase either the efficacy or potency of the molecule at its cognate receptor so that either fewer overall injections and/or less drug product would be required. As with many endogenously circulating peptides, GLP-1 already binds to the GLP-1R receptor with high affinity. However, there have been several studies aimed at elucidating the peptide–receptor interaction via both mutational and structural analyses in an effort to provide information to support rationale design of improved molecules. These have revealed that the N-terminal region is important for receptor activation while the C-terminal region has a greater contribution to binding. ((Siegel *et al.*, 1999; Xiao *et al.*, 2001; Green *et al.*, 2004). Stabilization of the α-helical regions in the N- and C-terminus using lactam bridges to create bi-cyclic GLP-1 analogues have been shown to enhance potency as well as render the molecule more resistant to degradation by the neutral endopeptidase NEP 24.11 (Murage *et al.*, 2008; 2010). Efforts have also been made to shorten the peptide from the native 30 amino acids of GLP-1. A novel class of 11 mer peptides consisting of a structurally modified 9 mer derived from the N-terminal region of GLP-1 linked to a substituted C-terminal biphenylalanine dipeptide exhibited enhanced pharmacokinetic profiles compared with GLP-1 (Mapelli *et al.*, 2009). However, these molecules will likely require further optimization in terms of their *in vivo* potency to be considered clinical candidates. Finally, efforts have attempted to extend half-life by synthesizing ester-based prodrug molecules that convert at extended rates to the parent GLP-1 analogue under physiological conditions due to their inherent chemical instability (De and DiMarchi, 2010). These efforts were successful at extending *in vitro* half-life (as measured by chemical stability) but it remains to be demonstrated whether these advantages translate *in vivo*.

With respect to amylin agonism increasing the efficacy or potency of the molecule at its cognate receptor is challenging for two reasons. First, as with GLP-1, amylin already binds its receptors in the pM range. Moreover, as discussed above, the amylin receptor complex is multidimensional (CTR + RAMP), and is not adequately captured in *in vitro* cell systems nor is the relative importance *in vivo* of various CTR + RAMP complexes involved in amylin's energy balance effects understood. Recently, we reviewed strategies towards developing improved amylin analogues (Roth *et al.*, 2008a). The helical region and the N-terminus of amylin were felt to represent the most promising regions for modifications. Our modified analogues were tested for binding in rat nucleus accumbens and decisions around selection of optimized analogues relied on *in vivo* efficacy and duration. This approach ultimately led to the development of a novel amylinomimetic, davalintide (AC2307). Davalintide was shown to bind with high affinity to amylin, calcitonin and calcitonin gene-related peptide receptors, yet suppress food intake for up to 23 h (compared with 6 h for native amylin administered at an equimolar dose (Mack *et al.*, 2010). Davalintide stimulated c-Fos expression studies as well as the dependence of an intact AP to observe anorexigenic effects suggested that the metabolic effects of davalintide and amylin are transduced via similar neuronal pathways. These findings suggest that with respect to amylin agonism, although difficult to extensively examine *in vitro*, it may be possible to design amylinomimetics with a longer duration of action that would require fewer daily injections.

Small molecule agonists/modulators

Although this review has focused on peptide-based therapeutics, small molecules with good bioavailability after oral administration remain attractive, patient-friendly options. Recent structural studies using photo-labelled probes confirmed that the amino terminal region of GLP-1 interacts with the amino terminal region of the receptor adjacent to the transmembrane domain as well as the 1st extracellular loop defining a pocket that could be targeted by small molecule agonists in the future (Chen *et al.*, 2010). Another approach that has been considered is to design allosteric modulators (e.g. molecules that bind at a site distinct from the endogenous ligand and that can impact ligand binding and/or modulate signalling pathways) of the GLP-1R (Christopoulos and Kenakin, 2002). Several of these molecules have been developed (Teng *et al.*, 2007; Irwin *et al.*, 2010; Sloop *et al.*, 2010). In theory these molecules can either be used alone or in combination with peptide ligands. An exciting aspect of some of these compounds is that they can selectively modulate various signalling pathways in a peptide-agonist-dependent manner highlighting the potential to develop molecules for distinct clinical efficacy (Koole *et al.*, 2010).

Phybrids/chimeras

The development path for a combination product faces several hurdles, both scientific and practical. Clinical development costs and complexity are increased by the need to compare the efficacy and safety of fixed dose combinations to the individual comparator arms. Further compounding these expenses is the likelihood that a combination product will require two separate manufacturing processes. In the case of injectable peptides, depending upon their compatibility for co-formulation, combinations will also likely require multiple daily injections. One way to mitigate some of these hurdles is to use chemical ligation strategies to link two molecules together into a single, novel chemical entity. Such peptide hybrids ('phybrids') would act as dual agonists by activating distinct receptors to induce separate but complementary pharmacological effects. Based on our observations that amylin and PYY(3–36) agonism exerted anorexigenic synergy and weight loss additivity (Roth *et al.*, 2007), we investigated whether this could be replicated in a proof-of-concept amylinomimetic : PYY(3–36) phybrid molecule. We demonstrated that single administration of the two parent molecules each elicited ~5% body weight loss in DIO rats over 1 week, their co-infusion induced ~12% weight loss, and the phybrid albeit at a somewhat higher dose, was able to approximate the same degree of weight loss (Roth *et al.*, 2010). The phybrid approach has also been applied to combined GLP-1 and amylin agonism (Mack *et al.*, 2008). When a single peptide entity composed of a GLP-1 agonist covalently attached to an amylinomimetic was assessed i*n vitro*, the GLP-1R : amylinomimetic phybrid stimulated cAMP through both the GLP-1 and the amylin/calcitonin receptors confirming each domain was functionally active, albeit with decreased potency for the CT receptor compared with the amylinomimetic parent. Twenty-eight days of SC infusion of the phybrid to Lep^{ob}/Lep^{ob} mice reduced haemoglobin A1c similar to the GLP-1R agonist parent component of the phybrid and in DIO rats reduced body weight comparably to the sum of the combination of the GLP-1R and amylinomimetic parent portions of the molecule, providing proof-of-concept for dual pharmacological action and anti-diabetic and anti-obesity properties in rodents. Another platform for combinations as single chemical entities involves co-agonist 'chimeric' molecules in which a single molecule can serve as an agonist at two (or more) distinct receptors. The chimeric platform is most applicable to partner molecules with sequence similarities. Recent examples include classes of compounds that agonize both GLP-1 and glucagon receptors. These molecules retained their *in vitro* potencies and improved glucose tolerance and reversed obesity in preclinical models (Day *et al.*, 2009; Pocai *et al.*, 2009). Future clinical studies on these novel classes of molecules are warranted.

GLP-1R and amylin agonism in other disease areas

In addition to metabolic disease, it is important to briefly note that both GLP-1R and amylin agonism may hold utility in other disease areas. The observations that GLP-1 agonism may play a role in pancreatic protection and regeneration coupled with the presence of GLP-1Rs in CNS regions implicated in neurodegenerative diseases (substantia nigra, subventricular zone, etc.) has led to the exploration and potential capitalization of GLP-1R stimulation in neuroprotection. The most mature of these areas of research involves salutary effects of GLP-1R agonism in multiple preclinical models of Parkinson's disease (e.g. 6-hydroxydopamine, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, lipopolysaccharide-induced) (Bertilsson *et al.*, 2008; Harkavyi *et al.*, 2008; Li *et al.*, 2009). Improvements are noted on a behavioural, biochemical and histological level and even link GLP-1R agonism to potential neurogenic effects [for recent reviews and discussion see (Harkavyi and Whitton, 2010; Perry and Greig, 2005)]. A similar body of research is emerging on beneficial effects of GLP-1 agonism in preclinical models of Alzheimer's disease (Li *et al.*, 2010; Hamilton *et al.*, 2011). The possibility that the pathology underlying neurodegenerative diseases is linked to common pathways and/or mechanisms has been raised given the increased link between the incidence of some of these neurodegenerative diseases in individuals with diabetes (Holst *et al.*, 2011).

Preclinical findings suggest that amylin agonism may hold utility in the treatment of neuropsychiatric disease. Amylin exerted anxiolytic and antidepressive effects in several preclinical assays (reviewed in Roth *et al.* (2009)). In a chronic stress model, the effects of amylin administration were assessed in rats that were given access to standard lab chow and sucrose solutions and were also exposed to daily restraint stress. Vehicle-treated rats increased their consumption of sucrose and increased visceral adiposity. By contrast, amylin-treated rats decreased their stress-induced sucrose consumption following restraint stress, while maintaining their intake of standard lab chow (Roth *et al.*, 2009). We also recently noted antidepressive and neurogenic potential of amylin agonism in estrogen-deficient animals. These studies were originally designed to explore the weight-lowering and metabolic effects of amylin in ovariectomized (OVX) and

intact (SHAM) animals (Trevaskis *et al*., 2010c). To our surprise, amylin was approximately twofold more efficacious in reducing body weight in OVX rats compared with SHAM as well as OVX control rats that received estradiol replacement therapy. In exploring the underlying mechanisms we found that amylin restored neurogenesis in the hippocampus of OVX rats and increased (approximately twofold) neurogenesis within the AP. These alterations were not evident in SHAM rats treated with amylin. Amylin-treated OVX rats also displayed decreased immobility (a depressed phenotype) as compared with SHAM-operated controls in the forced swim test. These findings warrant further exploration, but are noteworthy, as estradiol concentrations decrease in postmenopausal women who make up a high percentage of the obese population and incidences of depression are reported to increasing during menopause. To what extent amylin agonism may hold utility in neurodegenerative diseases remains to be elucidated.

Conclusions

The research reviewed herein on GLP-1 and amylin agonism represents a poignant example of how our understanding of the physiological actions and interactions and therapeutic potential of islet- and gut-hormones has grown dramatically in the past decades. In step with our deeper mechanistic understanding of these neurohormonal signals is progress in protein and peptide chemistry, formulation and drug delivery. The continued convergence of basic science and drug delivery efforts to develop mono- and combination-based peptide therapeutics that leverage this complex yet elegant physiology will hopefully yield important additions to the armamentarium for the treatment of metabolic disease/s.

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Conflict of interest

Jonathan D. Roth, Mary R. Erickson, Steve Chen and David G. Parkes are all employed by Amylin Pharmaceuticals, Inc. and own stock in Amylin Pharmaceuticals, Inc.

References

Abbott CR, Monteiro M, Small CJ, Sajedi A, Smith KL, Parkinson JR *et al.* (2005). The inhibitory effects of peripheral administration of peptide YY(3-36) and glucagon-like peptide-1 on food intake are attenuated by ablation of the vagal-brainstem-hypothalamic pathway. Brain Res 1044: 127–131.

Alvarez E, Martinez MD, Roncero I, Chowen JA, Garcia-Cuartero B, Gispert JD *et al.* (2005). The expression of GLP-1 receptor mRNA and protein allows the effect of GLP-1 on glucose metabolism in the human hypothalamus and brainstem. J Neurochem 92: 798–806.

Aronne L, Fujioka K, Aroda V, Chen K, Halseth A, Kesty NC *et al.* (2007). Progressive reduction in body weight after treatment with the amylin analog pramlintide in obese subjects: a phase 2, randomized, placebo-controlled, dose-escalation study. J Clin Endocrinol Metab 92: 2977–2983.

Asarian L, Eckel LA, Geary N (1998). Behaviorally specific inhibition of sham feeding by amylin. Peptides 19: 1711–1718.

Asmar M, Bache M, Knop FK, Madsbad S, Holst JJ (2010). Do the actions of glucagon-like peptide-1 on gastric emptying, appetite, and food intake involve release of amylin in humans? J Clin Endocrinol Metab 95: 2367–2375.

Ayala JE, Bracy DP, James FD, Burmeister MA, Wasserman DH, Drucker DJ (2010). Glucagon-like peptide-1 receptor knockout mice are protected from high-fat diet-induced insulin resistance. Endocrinology 151: 4678–4687.

Baggio LL, Huang Q, Brown TJ, Drucker DJ (2004). A recombinant human glucagon-like peptide (GLP)-1-albumin protein (albugon) mimics peptidergic activation of GLP-1 receptor-dependent pathways coupled with satiety, gastrointestinal motility, and glucose homeostasis. Diabetes 53: 2492–2500.

Baggio LL, Huang Q, Cao X, Drucker DJ (2008). An albumin-exendin-4 conjugate engages central and peripheral circuits regulating murine energy and glucose homeostasis. Gastroenterology 134: 1137–1147.

Baldo BA, Kelley AE (2001). Amylin infusion into rat nucleus accumbens potently depresses motor activity and ingestive behavior. Am J Physiol Regul Integr Comp Physiol 281: R1232–R1242.

Balkan B, Li X (2000). Portal GLP-1 administration in rats augments the insulin response to glucose via neuronal mechanisms. Am J Physiol Regul Integr Comp Physiol 279: R1449–R1454.

Banks WA, Kastin AJ (1998). Differential permeability of the blood-brain barrier to two pancreatic peptides: insulin and amylin. Peptides 19: 883–889.

Banks WA, Kastin AJ, Maness LM, Huang W, Jaspan JB (1995). Permeability of the blood-brain barrier to amylin. Life Sci 57: 1993–2001.

Baraboi ED, Smith P, Ferguson AV, Richard D (2010). Lesions of area postrema and subfornical organ alter exendin-4-induced brain activation without preventing the hypophagic effect of the GLP-1 receptor agonist. Am J Physiol Regul Integr Comp Physiol 298: R1098–R1110.

Barrera JG, D'Alessio DA, Drucker DJ, Woods SC, Seeley RJ (2009). Differences in the central anorectic effects of glucagon-like peptide-1 and exendin-4 in rats. Diabetes 58: 2820–2827.

Barth SW, Riediger T, Lutz TA, Rechkemmer G (2004). Peripheral amylin activates circumventricular organs expressing calcitonin receptor a/b subtypes and receptor-activity modifying proteins in the rat. Brain Res 997: 97–102.

Beaumont K, Kenney MA, Young AA, Rink TJ (1993). High affinity amylin binding sites in rat brain. Mol Pharmacol 44: 493–497.

Becskei C, Grabler V, Edwards GL, Riediger T, Lutz TA (2007). Lesion of the lateral parabrachial nucleus attenuates the anorectic effect of peripheral amylin and CCK. Brain Res 1162: 76–84.

Becskei C, Lutz TA, Riediger T (2009). Diet-derived nutrients mediate the inhibition of hypothalamic NPY neurons in the arcuate nucleus of mice during refeeding. Am J Physiol Regul Integr Comp Physiol 297: R100–R110.

Bello NT, Kemm MH, Ofeldt EM, Moran TH (2010). Dose combinations of exendin-4 and salmon calcitonin produce additive and synergistic reductions in food intake in nonhuman primates. Am J Physiol Regul Integr Comp Physiol 299: R945–R952.

Bertilsson G, Patrone C, Zachrisson O, Andersson A, Dannaeus K, Heidrich J *et al*. (2008). Peptide hormone exendin-4 stimulates subventricular zone neurogenesis in the adult rodent brain and induces recovery in an animal model of Parkinson's disease. J Neurosci Res 86: 326–338.

Bhavsar S, Watkins J, Young A (1998). Synergy between amylin and cholecystokinin for inhibition of food intake in mice. Physiol Behav 64: 557–561.

Bjerre Knudsen L, Madsen LW, Andersen S, Almholt K, de Boer AS, Drucker DJ *et al*. (2010). Glucagon-like Peptide-1 receptor agonists activate rodent thyroid C-cells causing calcitonin release and C-cell proliferation. Endocrinology 151: 1473–1486.

Bojanowska E, Nowak A (2007). Interactions between leptin and exendin-4, a glucagon-like peptide-1 agonist, in the regulation of food intake in the rat. J Physiol Pharmacol 58: 349–360.

Brennan IM, Feltrin KL, Horowitz M, Smout AJ, Meyer JH, Wishart J *et al*. (2005). Evaluation of interactions between CCK and GLP-1 in their effects on appetite, energy intake, and antropyloroduodenal motility in healthy men. Am J Physiol Regul Integr Comp Physiol 288: R1477–R1485.

Bullock BP, Heller RS, Habener JF (1996). Tissue distribution of messenger ribonucleic acid encoding the rat glucagon-like peptide-1 receptor. Endocrinology 137: 2968–2978.

Campos RV, Lee YC, Drucker DJ (1994). Divergent tissue-specific and developmental expression of receptors for glucagon and glucagon-like peptide-1 in the mouse. Endocrinology 134: 2156–2164.

Cegla J, Tan TM, Bloom SR (2010). Gut-brain cross-talk in appetite regulation. Curr Opin Clin Nutr Metab Care 13: 588–593.

Chance WT, Balasubramaniam A, Chen X, Fischer JE (1992). Tests of adipsia and conditioned taste aversion following the intrahypothalamic injection of amylin. Peptides 13: 961–964.

Chen Q, Pinon DI, Miller LJ, Dong M (2010). Spatial approximations between residues 6 and 12 in the amino-terminal region of glucagon-like peptide 1 and its receptor: a region critical for biological activity. J Biol Chem 285: 24508–24518.

Christensen M, Knop FK (2010). Once-weekly GLP-1 agonists: how do they differ from exenatide and liraglutide? Curr Diab Rep 10: 124–132.

Christopoulos A, Kenakin T (2002). G protein-coupled receptor allosterism and complexing. Pharmacol Rev 54: 323–374.

Colburn WA, Gottlieb AB, Koda J, Kolterman OG (1996). Pharmacokinetics and pharmacodynamics of AC137 (25,28,29 tripro-amylin, human) after intravenous bolus and infusion doses in patients with insulin-dependent diabetes. J Clin Pharmacol 36: 13–24.

Davey RA, Turner AG, McManus JF, Chiu WS, Tjahyono F, Moore AJ *et al*. (2008). Calcitonin receptor plays a physiological role to protect against hypercalcemia in mice. J Bone Miner Res 23: 1182–1193.

Day JW, Ottaway N, Patterson JT, Gelfanov V, Smiley D, Gidda J *et al*. (2009). A new glucagon and GLP-1 co-agonist eliminates obesity in rodents. Nat Chem Biol 5: 749–757.

De A, DiMarchi RD (2010). Synthesis and characterization of ester-based prodrugs of glucagon-like peptide 1. Biopolymers 94: 448–456.

Deane AM, Nguyen NQ, Stevens JE, Fraser RJ, Holloway RH, Besanko LK *et al*. (2010). Endogenous glucagon-like peptide-1 slows gastric emptying in healthy subjects, attenuating postprandial glycemia. J Clin Endocrinol Metab 95: 215–221.

Dillon JS, Tanizawa Y, Wheeler MB, Leng XH, Ligon BB, Rabin DU *et al*. (1993). Cloning and functional expression of the human glucagon-like peptide-1 (GLP-1) receptor. Endocrinology 133: 1907–1910.

Ding WG, Renstrom E, Rorsman P, Buschard K, Gromada J (1997). Glucagon-like peptide I and glucose-dependent insulinotropic polypeptide stimulate Ca2+-induced secretion in rat alpha-cells by a protein kinase A-mediated mechanism. Diabetes 46: 792–800.

Doyle ME, Egan JM (2007). Mechanisms of action of glucagon-like peptide 1 in the pancreas. Pharmacol Ther 113: 546–593.

Egan JM, Montrose-Rafizadeh C, Wang Y, Bernier M, Roth J (1994). Glucagon-like peptide-1(7-36) amide (GLP-1) enhances insulin-stimulated glucose metabolism in 3T3-L1 adipocytes: one of several potential extrapancreatic sites of GLP-1 action. Endocrinology 135: 2070–2075.

Field BC, Chaudhri OB, Bloom SR (2009). Obesity treatment: novel peripheral targets. Br J Clin Pharmacol 68: 830–843.

Flamez D, Gilon P, Moens K, Van Breusegem A, Delmeire D, Scrocchi LA *et al*. (1999). Altered cAMP and Ca2+ signaling in mouse pancreatic islets with glucagon-like peptide-1 receptor null phenotype. Diabetes 48: 1979–1986.

Gebre-Medhin S, Mulder H, Pekny M, Westermark G, Tornell J, Westermark P *et al*. (1998). Increased insulin secretion and glucose tolerance in mice lacking islet amyloid polypeptide (amylin). Biochem Biophys Res Commun 250: 271–277.

Gedulin BR, Rink TJ, Young AA (1997). Dose-response for glucagonostatic effect of amylin in rats. Metabolism 46: 67–70.

Graziano MP, Hey PJ, Borkowski D, Chicchi GG, Strader CD (1993). Cloning and functional expression of a human glucagon-like peptide-1 receptor. Biochem Biophys Res Commun 196: 141–146.

Green BD, Gault VA, O'Harte FP, Flatt PR (2004). Structurally modified analogues of glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) as future antidiabetic agents. Curr Pharm Des 10: 3651–3662.

Gromada J, Rorsman P (2004). New insights into the regulation of glucagon secretion by glucagon-like peptide-1. Horm Metab Res 36: 822–829.

Gromada J, Brock B, Schmitz O, Rorsman P (2004). Glucagon-like peptide-1: regulation of insulin secretion and therapeutic potential. Basic Clin Pharmacol Toxicol 95: 252–262.

Gutzwiller JP, Degen L, Matzinger D, Prestin S, Beglinger C (2004). Interaction between GLP-1 and CCK-33 in inhibiting food intake and appetite in men. Am J Physiol Regul Integr Comp Physiol 287: R562–R567.

Hamilton A, Patterson S, Porter D, Gault VA, Holscher C (2011). Novel GLP-1 mimetics developed to treat type 2 diabetes promote progenitor cell proliferation in the brain. J Neurosci Res 89: 481–489.

Hansotia T, Drucker DJ (2005). GIP and GLP-1 as incretin hormones: lessons from single and double incretin receptor knockout mice. Regul Pept 128: 125–134.

Hansotia T, Baggio LL, Delmeire D, Hinke SA, Yamada Y, Tsukiyama K *et al*. (2004). Double incretin receptor knockout (DIRKO) mice reveal an essential role for the enteroinsular axis in transducing the glucoregulatory actions of DPP-IV inhibitors. Diabetes 53: 1326–1335.

Harkavyi A, Whitton PS (2010). Glucagon-like peptide 1 receptor stimulation as a means of neuroprotection. Br J Pharmacol 159: 495–501.

Harkavyi A, Abuirmeileh A, Lever R, Kingsbury AE, Biggs CS, Whitton PS (2008). Glucagon-like peptide 1 receptor stimulation reverses key deficits in distinct rodent models of Parkinson's disease. J Neuroinflammation 5: 5–19.

Hayashi Y, Yamamoto M, Mizoguchi H, Watanabe C, Ito R, Yamamoto S *et al*. (2009). Mice deficient for glucagon gene-derived peptides display normoglycemia and hyperplasia of islet {alpha}-cells but not of intestinal L-cells. Mol Endocrinol 23: 1990–1999.

de Heer J, Rasmussen C, Coy DH, Holst JJ (2008). Glucagon-like peptide-1, but not glucose-dependent insulinotropic peptide, inhibits glucagon secretion via somatostatin (receptor subtype 2) in the perfused rat pancreas. Diabetologia 51: 2263–2270.

Hollander P, Maggs DG, Ruggles JA, Fineman M, Shen L, Kolterman OG *et al*. (2004). Effect of pramlintide on weight in overweight and obese insulin-treated type 2 diabetes patients. Obes Res 12: 661–668.

Holmes GM, Browning KN, Tong M, Qualls-Creekmore E, Travagli RA (2009). Vagally mediated effects of glucagon-like peptide 1: in vitro and in vivo gastric actions. J Physiol 587: 4749–4759.

Holst JJ (2007). The physiology of glucagon-like peptide 1. Physiol Rev 87: 1409–1439.

Holst JJ, Burcelin R, Nathanson E (2011). Neuroprotective properties of GLP-1: theoretical and practical applications. Curr Med Res Opin 27: 547–558.

Irwin N, Flatt PR, Patterson S, Green BD (2010). Insulin-releasing and metabolic effects of small molecule GLP-1 receptor agonist 6,7-dichloro-2-methylsulfonyl-3-N-tert-butylaminoquinoxaline. Eur J Pharmacol 628: 268–273.

Kastin AJ, Akerstrom V, Pan W (2002). Interactions of glucagon-like peptide-1 (GLP-1) with the blood-brain barrier. J Mol Neurosci 18: 7–14.

Kinzig KP, D'Alessio DA, Seeley RJ (2002). The diverse roles of specific GLP-1 receptors in the control of food intake and the response to visceral illness. J Neurosci 22: 10470–10476.

Klopfenstein BK, Krisky C, Rooney W, Purnell JQ (2010). Amygdala activation by leptin predicts food intake in lean men. In: *Late Breaking Abstraction – The Obesity Society*.

Koda JE, Fineman M, Rink TJ, Dailey GE, Muchmore DB, Linarelli LG (1992). Amylin concentrations and glucose control. Lancet 339: 1179–1180.

Koole C, Wootten D, Simms J, Valant C, Sridhar R, Woodman OL *et al*. (2010). Allosteric ligands of the glucagon-like peptide 1 receptor (GLP-1R) differentially modulate endogenous and exogenous peptide responses in a pathway-selective manner: implications for drug screening. Mol Pharmacol 78: 456–465.

Korner M, Stockli M, Waser B, Reubi JC (2007). GLP-1 receptor expression in human tumors and human normal tissues: potential for in vivo targeting. J Nucl Med 48: 736–743.

Larsen PJ, Tang-Christensen M, Holst JJ, Orskov C (1997). Distribution of glucagon-like peptide-1 and other preproglucagon-derived peptides in the rat hypothalamus and brainstem. Neuroscience 77: 257–270.

Li N, Fang CY, Wang ZZ, Wang YL, Wang FB, Gao E *et al*. (2004). Expression of calcitonin gene-related peptide type 1 receptor mRNA and their activity-modifying proteins in the rat nucleus accumbens. Neurosci Lett 362: 146–149.

Li Y, Perry T, Kindy MS, Harvey BK, Tweedie D, Holloway HW *et al*. (2009). GLP-1 receptor stimulation preserves primary cortical and dopaminergic neurons in cellular and rodent models of stroke and Parkinsonism. Proc Natl Acad Sci USA 106: 1285–1290.

Li Y, Duffy KB, Ottinger MA, Ray B, Bailey JA, Holloway HW *et al*. (2010). GLP-1 receptor stimulation reduces amyloid-beta peptide accumulation and cytotoxicity in cellular and animal models of Alzheimer's disease. J Alzheimers Dis 19: 1205–1219.

Ling Z, Wu D, Zambre Y, Flamez D, Drucker DJ, Pipeleers DG *et al*. (2001). Glucagon-like peptide 1 receptor signaling influences topography of islet cells in mice. Virchows Arch 438: 382–387.

Lund PK, Goodman RH, Dee PC, Habener JF (1982). Pancreatic preproglucagon cDNA contains two glucagon-related coding sequences arranged in tandem. Proc Natl Acad Sci USA 79: 345–349.

Lutz TA, Del Prete E, Scharrer E (1994). Reduction of food intake in rats by intraperitoneal injection of low doses of amylin. Physiol Behav 55: 891–895.

Lutz TA, Del Prete E, Scharrer E (1995). Subdiaphragmatic vagotomy does not influence the anorectic effect of amylin. Peptides 16: 457–462.

Lutz TA, Althaus J, Rossi R, Scharrer E (1998a). Anorectic effect of amylin is not transmitted by capsaicin-sensitive nerve fibers. Am J Physiol 274 (Part 2): R1777–R1782.

Lutz TA, Senn M, Althaus J, Del Prete E, Ehrensperger F, Scharrer E (1998b). Lesion of the area postrema/nucleus of the solitary tract (AP/NTS) attenuates the anorectic effects of amylin and calcitonin gene-related peptide (CGRP) in rats. Peptides 19: 309–317.

Mack C, Wilson J, Athanacio J, Reynolds J, Laugero K, Guss S *et al*. (2007). Pharmacological actions of the peptide hormone amylin in the long-term regulation of food intake, food preference, and body weight. Am J Physiol Regul Integr Comp Physiol 293: R1855–R1863.

Mack C, Wilson J, Gu G, Cole R, Roland R, Vu C *et al*. (2008). Central activation and weight-lowering actions of AC164209, a peptide hybrid linking a glucagon-like peptide-1 (GLP-1) receptor agonist and an amylin mimetic. The Obesity Society, P-489.

Mack CM, Soares CJ, Wilson JK, Athanacio JR, Turek VF, Trevaskis JL *et al*. (2010). Davalintide (AC2307), a novel amylin-mimetic peptide: enhanced pharmacological properties over native amylin to reduce food intake and body weight. Int J Obes (Lond) 34: 385–395.

McLatchie LM, Fraser NJ, Main MJ, Wise A, Brown J, Thompson N *et al*. (1998). RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor. Nature 393: 333–339.

Madsbad S, Kielgast U, Asmar M, Deacon C, Torekov SS, Holst JJ (2011). An overview of once-weekly GLP-1 receptor agonists - available efficacy and safety data and perspectives for the future. Diabetes Obes Metab 13: 394–407.

Malone J, Trautmann M, Wilhelm K, Taylor K, Kendall DM (2009). Exenatide once weekly for the treatment of type 2 diabetes. Expert Opin Investig Drugs 18: 359–367.

Mapelli C, Natarajan SI, Meyer JP, Bastos MM, Bernatowicz MS, Lee VG *et al*. (2009). Eleven amino acid glucagon-like peptide-1 receptor agonists with antidiabetic activity. J Med Chem 52: 7788–7799.

Marino MT, Costello D, Baughman R, Boss A, Cassidy J, Damico C *et al*. (2010). Pharmacokinetics and pharmacodynamics of inhaled GLP-1 (MKC253): proof-of-concept studies in healthy normal volunteers and in patients with type 2 diabetes. Clin Pharmacol Ther 88: 243–250.

Mentlein R, Gallwitz B, Schmidt WE (1993). Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7-36)amide, peptide histidine methionine and is responsible for their degradation in human serum. Eur J Biochem 214: 829–835.

Merchenthaler I, Lane M, Shughrue P (1999). Distribution of pre-pro-glucagon and glucagon-like peptide-1 receptor messenger RNAs in the rat central nervous system. J Comp Neurol 403: 261–280.

Mollet A, Gilg S, Riediger T, Lutz TA (2004). Infusion of the amylin antagonist AC 187 into the area postrema increases food intake in rats. Physiol Behav 81: 149–155.

Morley JE, Suarez MD, Mattamal M, Flood JF (1997). Amylin and food intake in mice: effects on motivation to eat and mechanism of action. Pharmacol Biochem Behav 56: 123–129.

Mortensen K, Christensen LL, Holst JJ, Orskov C (2003). GLP-1 and GIP are colocalized in a subset of endocrine cells in the small intestine. Regul Pept 114: 189–196.

Morton GJ, Cummings DE, Baskin DG, Barsh GS, Schwartz MW (2006). Central nervous system control of food intake and body weight. Nature 443: 289–295.

Mulder H, Gebre-Medhin S, Betsholtz C, Sundler F, Ahren B (2000). Islet amyloid polypeptide (amylin)-deficient mice develop a more severe form of alloxan-induced diabetes. Am J Physiol Endocrinol Metab 278: E684–E691.

Murage EN, Schroeder JC, Beinborn M, Ahn JM (2008). Search for alpha-helical propensity in the receptor-bound conformation of glucagon-like peptide-1. Bioorg Med Chem 16: 10106–10112.

Murage EN, Gao G, Bisello A, Ahn JM (2010). Development of potent glucagon-like peptide-1 agonists with high enzyme stability via introduction of multiple lactam bridges. J Med Chem 53: 6412–6420.

Nagell CF, Wettergren A, Orskov C, Holst JJ (2006). Inhibitory effect of GLP-1 on gastric motility persists after vagal deafferentation in pigs. Scand J Gastroenterol 41: 667–672.

Nakabayashi H, Nishizawa M, Nakagawa A, Takeda R, Niijima A (1996). Vagal hepatopancreatic reflex effect evoked by intraportal appearance of tGLP-1. Am J Physiol 271 (Part 1): E808–E813.

Nathan Y, Lee A, Evans T, Coates S, Reed LJ, Brammer MJ *et al*. (2010). Acute administration of the GLP-1 receptor agonist exenatide restores impaired central responses to food ingestion in type 2 diabetes. In: *European Association For The Study of Diabetes*.

Nauck MA, Heimesaat MM, Behle K, Holst JJ, Nauck MS, Ritzel R *et al*. (2002). Effects of glucagon-like peptide 1 on counterregulatory hormone responses, cognitive functions, and insulin secretion during hyperinsulinemic, stepped hypoglycemic clamp experiments in healthy volunteers. J Clin Endocrinol Metab 87: 1239–1246.

Neary NM, Small CJ, Druce MR, Park AJ, Ellis SM, Semjonous NM *et al*. (2005). Peptide YY3-36 and glucagon-like peptide-17-36 inhibit food intake additively. Endocrinology 146: 5120–5127.

Nicolaus M, Brodl J, Linke R, Woerle HJ, Goke B, Schirra J (2011). Endogenous GLP-1 regulates postprandial glycemia in humans: relative contributions of insulin, glucagon, and gastric emptying. J Clin Endocrinol Metab 96: 229–236.

Nielsen LL, Young AA, Parkes DG (2004). Pharmacology of exenatide (synthetic exendin-4): a potential therapeutic for improved glycemic control of type 2 diabetes. Regul Pept 117: 77–88.

Ogawa A, Harris V, McCorkle SK, Unger RH, Luskey KL (1990). Amylin secretion from the rat pancreas and its selective loss after streptozotocin treatment. J Clin Invest 85: 973–976.

Orskov C, Poulsen SS, Moller M, Holst JJ (1996). Glucagon-like peptide I receptors in the subfornical organ and the area postrema are accessible to circulating glucagon-like peptide I. Diabetes 45: 832–835.

Osto M, Wielinga PY, Alder B, Walser N, Lutz TA (2007). Modulation of the satiating effect of amylin by central ghrelin, leptin and insulin. Physiol Behav 91: 566–572.

Pannacciulli N, Le DS, Salbe AD, Chen K, Reiman EM, Tataranni PA *et al*. (2007). Postprandial glucagon-like peptide-1 (GLP-1) response is positively associated with changes in neuronal activity of brain areas implicated in satiety and food intake regulation in humans. Neuroimage 35: 511–517.

Parthier C, Reedtz-Runge S, Rudolph R, Stubbs MT (2009). Passing the baton in class B GPCRs: peptide hormone activation via helix induction? Trends Biochem Sci 34: 303–310.

Perry T, Greig NH (2005). Enhancing central nervous system endogenous GLP-1 receptor pathways for intervention in Alzheimer's disease. Curr Alzheimer Res 2: 377–385.

Pocai A, Carrington PE, Adams JR, Wright M, Eiermann G, Zhu L *et al*. (2009). Glucagon-like peptide 1/glucagon receptor dual agonism reverses obesity in mice. Diabetes 58: 2258–2266.

Potes CS, Lutz TA (2010). Brainstem mechanisms of amylin-induced anorexia. Physiol Behav 100: 511–518.

Potes CS, Turek VF, Cole RL, Vu C, Roland BL, Roth JD *et al*. (2010). Noradrenergic neurons of the area postrema mediate amylin's hypophagic action. Am J Physiol Regul Integr Comp Physiol 299: R623–R631.

Poyner DR, Sexton PM, Marshall I, Smith DM, Quirion R, Born W *et al*. (2002). International Union of Pharmacology. XXXII. The mammalian calcitonin gene-related peptides, adrenomedullin, amylin, and calcitonin receptors. Pharmacol Rev 54: 233–246.

Ravussin E, Smith SR, Mitchell JA, Shringarpure R, Shan K, Maier H *et al*. (2009). Enhanced weight loss with pramlintide/metreleptin: an integrated neurohormonal approach to obesity pharmacotherapy. Obesity (Silver Spring) 17: 1736–1743.

Reidelberger RD, Haver AC, Apenteng BA, Anders KL, Steenson SM (2011). Effects of exendin-4 alone and with peptide YY(3-36) on food intake and body weight in diet-induced obese rats. Obesity (Silver Spring) 19: 121–127.

Riediger T, Schmid HA, Lutz T, Simon E (2001). Amylin potently activates AP neurons possibly via formation of the excitatory second messenger cGMP. Am J Physiol Regul Integr Comp Physiol 281: R1833–R1843.

Riediger T, Zuend D, Becskei C, Lutz TA (2004). The anorectic hormone amylin contributes to feeding-related changes of neuronal activity in key structures of the gut-brain axis. Am J Physiol Regul Integr Comp Physiol 286: R114–R122.

Rocca AS, Brubaker PL (1999). Role of the vagus nerve in mediating proximal nutrient-induced glucagon-like peptide-1 secretion. Endocrinology 140: 1687–1694.

Roth JD, Hughes H, Kendall E, Baron AD, Anderson CM (2006). Antiobesity effects of the beta-cell hormone amylin in diet-induced obese rats: effects on food intake, body weight, composition, energy expenditure, and gene expression. Endocrinology 147: 5855–5864.

Roth JD, Coffey T, Jodka CM, Maier H, Athanacio JR, Mack CM *et al.* (2007). Combination therapy with amylin and peptide YY[3-36] in obese rodents: anorexigenic synergy and weight loss additivity. Endocrinology 148: 6054–6061.

Roth JD, Mack CM, Soares CJ, Ghosh SS, Parkes DG (2008a). Amylin-Based Pharmacotherapy: past, present and future. Immun Endoc & Metab Agents Med Chem 8: 317–324.

Roth JD, Roland BL, Cole RL, Trevaskis JL, Weyer C, Koda JE *et al.* (2008b). Leptin responsiveness restored by amylin agonism in diet-induced obesity: evidence from nonclinical and clinical studies. Proc Natl Acad Sci USA 105: 7257–7262.

Roth JD, Maier H, Chen S, Roland BL (2009). Implications of amylin receptor agonism: integrated neurohormonal mechanisms and therapeutic applications. Arch Neurol 66: 306–310.

Roth JD, Trevaskis JL, Turek VF, Parkes DG (2010). 'Weighing in' on synergy: preclinical research on neurohormonal anti-obesity combinations. Brain Res 1350: 86–94.

Rowland NE, Richmond RM (1999). Area postrema and the anorectic actions of dexfenfluramine and amylin. Brain Res 820: 86–91.

Rowland NE, Crews EC, Gentry RM (1997). Comparison of Fos induced in rat brain by GLP-1 and amylin. Regul Pept 71: 171–174.

Rushing PA, Seeley RJ, Air EL, Lutz TA, Woods SC (2002). Acute 3rd-ventricular amylin infusion potently reduces food intake but does not produce aversive consequences. Peptides 23: 985–988.

Ruttimann EB, Arnold M, Hillebrand JJ, Geary N, Langhans W (2009). Intrameal hepatic portal and intraperitoneal infusions of glucagon-like peptide-1 reduce spontaneous meal size in the rat via different mechanisms. Endocrinology 150: 1174–1181.

Salehi M, Vahl TP, D'Alessio DA (2008). Regulation of islet hormone release and gastric emptying by endogenous glucagon-like peptide 1 after glucose ingestion. J Clin Endocrinol Metab 93: 4909–4916.

Sandhu H, Wiesenthal SR, MacDonald PE, McCall RH, Tchipashvili V, Rashid S *et al.* (1999). Glucagon-like peptide 1 increases insulin sensitivity in depancreatized dogs. Diabetes 48: 1045–1053.

Scrocchi LA, Drucker DJ (1998). Effects of aging and a high fat diet on body weight and glucose tolerance in glucagon-like peptide-1 receptor -/- mice. Endocrinology 139: 3127–3132.

Scrocchi LA, Brown TJ, MaClusky N, Brubaker PL, Auerbach AB, Joyner AL *et al.* (1996). Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide 1 receptor gene. Nat Med 2: 1254–1258.

Scrocchi LA, Brown TJ, Drucker DJ (1997). Leptin sensitivity in nonobese glucagon-like peptide I receptor -/- mice. Diabetes 46: 2029–2034.

Seeley RJ, Blake K, Rushing PA, Benoit S, Eng J, Woods SC *et al.* (2000). The role of CNS glucagon-like peptide-1 (7-36) amide receptors in mediating the visceral illness effects of lithium chloride. J Neurosci 20: 1616–1621.

Seo S, Ju S, Chung H, Lee D, Park S (2008). Acute effects of glucagon-like peptide-1 on hypothalamic neuropeptide and AMP activated kinase expression in fasted rats. Endocr J 55: 867–874.

Seth R, Knight WD, Overton JM (2010). Combined amylin-leptin treatment lowers blood pressure and adiposity in lean and obese rats. Int J Obes (Lond) DOI: 10.1038/ijo.2010.262.

Sexton PM, Poyner DR, Simms J, Christopoulos A, Hay DL (2009). Modulating receptor function through RAMPs: can they represent drug targets in themselves? Drug Discov Today 14: 413–419.

Shin YK, Martin B, Golden E, Dotson CD, Maudsley S, Kim W *et al.* (2008). Modulation of taste sensitivity by GLP-1 signaling. J Neurochem 106: 455–463.

Siegel EG, Gallwitz B, Scharf G, Mentlein R, Morys-Wortmann C, Folsch UR *et al.* (1999). Biological activity of GLP-1-analogues with N-terminal modifications. Regul Pept 79: 93–102.

Silvestre RA, Rodriguez-Gallardo J, Jodka C, Parkes DG, Pittner RA, Young AA *et al.* (2001). Selective amylin inhibition of the glucagon response to arginine is extrinsic to the pancreas. Am J Physiol Endocrinol Metab 280: E443–E449.

Skibicka KP, Alhadeff AL, Grill HJ (2009). Hindbrain cocaine- and amphetamine-regulated transcript induces hypothermia mediated by GLP-1 receptors. J Neurosci 29: 6973–6981.

Sloop KW, Willard FS, Brenner MB, Ficorilli J, Valasek K, Showalter AD *et al.* (2010). Novel small molecule glucagon-like peptide-1 receptor agonist stimulates insulin secretion in rodents and from human islets. Diabetes 59: 3099–3107.

Smith SR, Blundell JE, Burns C, Ellero C, Schroeder BE, Kesty NC *et al.* (2007). Pramlintide treatment reduces 24-h caloric intake and meal sizes and improves control of eating in obese subjects: a 6-wk translational research study. Am J Physiol Endocrinol Metab 293: E620–E627.

Steinert RE, Poller B, Castelli MC, Drewe J, Beglinger C (2010). Oral administration of glucagon-like peptide 1 or peptide YY 3-36 affects food intake in healthy male subjects. Am J Clin Nutr 92: 810–817.

Talsania T, Anini Y, Siu S, Drucker DJ, Brubaker PL (2005). Peripheral exendin-4 and peptide YY(3-36) synergistically reduce food intake through different mechanisms in mice. Endocrinology 146: 3748–3756.

Tang-Christensen M, Cowley MA (2007). GLP-1 analogs: satiety without malaise? Am J Physiol Regul Integr Comp Physiol 293: R981–R982.

Tang-Christensen M, Vrang N, Larsen PJ (1998). Glucagon-like peptide 1(7-36) amide's central inhibition of feeding and peripheral inhibition of drinking are abolished by neonatal monosodium glutamate treatment. Diabetes 47: 530–537.

Teng M, Johnson MD, Thomas C, Kiel D, Lakis JN, Kercher T *et al.* (2007). Small molecule ago-allosteric modulators of the human glucagon-like peptide-1 (hGLP-1) receptor. Bioorg Med Chem Lett 17: 5472–5478.

Thiele TE, Van Dijk G, Campfield LA, Smith FJ, Burn P, Woods SC *et al.* (1997). Central infusion of GLP-1, but not leptin, produces conditioned taste aversions in rats. Am J Physiol 272 (Part 2): R726–R730.

Thorens B (1992). Expression cloning of the pancreatic beta cell receptor for the gluco-incretin hormone glucagon-like peptide 1. Proc Natl Acad Sci USA 89: 8641–8645.

Thorens B, Porret A, Buhler L, Deng SP, Morel P, Widmann C (1993). Cloning and functional expression of the human islet GLP-1 receptor. Demonstration that exendin-4 is an agonist and exendin-(9-39) an antagonist of the receptor. Diabetes 42: 1678–1682.

Tomas E, Stanojevic V, Habener JF (2011). GLP-1-derived nonapeptide GLP-1(28-36)amide targets to mitochondria and suppresses glucose production and oxidative stress in isolated mouse hepatocytes. Regul Pept 169: 43–48.

Trevaskis JL, Coffey T, Cole R, Lei C, Wittmer C, Walsh B *et al*. (2008). Amylin-mediated restoration of leptin responsiveness in diet-induced obesity: magnitude and mechanisms. Endocrinology 149: 5679–5687.

Trevaskis JL, Parkes DG, Roth JD (2010a). Insights into amylin-leptin synergy. Trends Endocrinol Metab 21: 473–479.

Trevaskis JL, Turek VF, Griffin PS, Wittmer C, Parkes DG, Roth JD (2010b). Multi-hormonal weight loss combinations in diet-induced obese rats: therapeutic potential of cholecystokinin? Physiol Behav 100: 187–195.

Trevaskis JL, Turek VF, Wittmer C, Griffin PS, Wilson JK, Reynolds JM *et al*. (2010c). Enhanced amylin-mediated body weight loss in estradiol-deficient diet-induced obese rats. Endocrinology 151: 5657–5668.

Turek VF, Trevaskis JL, Levin BE, Dunn-Meynell AA, Irani B, Gu G *et al*. (2010). Mechanisms of amylin/leptin synergy in rodent models. Endocrinology 151: 143–152.

Turner AG, Tjahyono F, Chiu WS, Skinner J, Sawyer R, Moore AJ *et al*. (2011). The role of the calcitonin receptor in protecting against induced hypercalcemia is mediated via its actions in osteoclasts to inhibit bone resorption. Bone 48: 354–361.

Unger RH, Orci L (2010). Paracrinology of islets and the paracrinopathy of diabetes. Proc Natl Acad Sci USA 107: 16009–16012.

Vahl TP, Tauchi M, Durler TS, Elfers EE, Fernandes TM, Bitner RD *et al*. (2007). Glucagon-like peptide-1 (GLP-1) receptors expressed on nerve terminals in the portal vein mediate the effects of endogenous GLP-1 on glucose tolerance in rats. Endocrinology 148: 4965–4973.

Vine W, Smith P, LaChappell R, Blase E, Lumpkin R, Young A (1998). Nephrectomy decreases amylin and pramlintide clearance in rats. Horm Metab Res 30: 514–517.

Wan S, Coleman FH, Travagli RA (2007). Glucagon-like peptide-1 excites pancreas-projecting preganglionic vagal motoneurons. Am J Physiol Gastrointest Liver Physiol 292: G1474–G1482.

Wettergren A, Wojdemann M, Meisner S, Stadil F, Holst JJ (1997). The inhibitory effect of glucagon-like peptide-1 (GLP-1) 7-36 amide on gastric acid secretion in humans depends on an intact vagal innervation. Gut 40: 597–601.

Weyer C, Maggs DG, Young AA, Kolterman OG (2001). Amylin replacement with pramlintide as an adjunct to insulin therapy in type 1 and type 2 diabetes mellitus: a physiological approach toward improved metabolic control. Curr Pharm Des 7: 1353–1373.

Wickbom J, Herrington MK, Permert J, Jansson A, Arnelo U (2008). Gastric emptying in response to IAPP and CCK in rats with subdiaphragmatic afferent vagotomy. Regul Pept 148: 21–25.

Williams DL (2009). Minireview: finding the sweet spot: peripheral versus central glucagon-like peptide 1 action in feeding and glucose homeostasis. Endocrinology 150: 2997–3001.

Williams DL, Baskin DG, Schwartz MW (2006). Leptin regulation of the anorexic response to glucagon-like peptide-1 receptor stimulation. Diabetes 55: 3387–3393.

Williams DL, Baskin DG, Schwartz MW (2009). Evidence that intestinal glucagon-like peptide-1 plays a physiological role in satiety. Endocrinology 150: 1680–1687.

Witte AB, Gryback P, Jacobsson H, Naslund E, Hellstrom PM, Holst JJ *et al*. (2011). Involvement of endogenous glucagon-like peptide-1 in regulation of gastric motility and pancreatic endocrine secretion. Scand J Gastroenterol 46: 428–435.

World Health Organization (2011) http://www.who.int/diabetes/facts/world_figures/en/index.html. Prevalence of Diabetes Worldwide. In: *World Health Organization*.

Xiao Q, Giguere J, Parisien M, Jeng W, St-Pierre SA, Brubaker PL *et al*. (2001). Biological activities of glucagon-like peptide-1 analogues in vitro and in vivo. Biochemistry 40: 2860–2869.

Yamamoto H, Kishi T, Lee CE, Choi BJ, Fang H, Hollenberg AN *et al*. (2003). Glucagon-like peptide-1-responsive catecholamine neurons in the area postrema link peripheral glucagon-like peptide-1 with central autonomic control sites. J Neurosci 23: 2939–2946.

Young A (2005a). Clinical studies. Adv Pharmacol 52: 289–320.

Young A (2005b). Historical background. Adv Pharmacol 52: 1–18.

Young A (2005c). Inhibition of gastric emptying. Adv Pharmacol 52: 99–121.

Young A (2005d). Inhibition of insulin secretion. Adv Pharmacol 52: 173–192.

Zhang Z, Liu X, Morgan DA, Kuburas A, Thedens DR, Russo AF *et al*. (2011). Neuronal Receptor Activity Modifying Protein-1 Promotes Energy Expenditure in Mice. Diabetes 60: 1063–1071.