BJP British Journal of Pharmacology

DOI:10.1111/bph.12313
www.brjpharmacol.org

Themed Section: Molecular Pharmacology of GPCRs

REVIEW

Molecular mechanisms underlying physiological and receptor pleiotropic effects mediated by GLP-1R activation

K Pabreja, M A Mohd, C Koole, D Wootten and S G B Furness

Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Vic., Australia

Correspondence
Sebastian George Barton Furness, Drug Discovery Biology Laboratory Monash Institute of Pharmaceutical Sciences Monash University, 399 Royal Parade, Parkville, Vic. 3052, Australia. E-mail: sebastian.furness@monash.edu

Keywords
type 2 diabetes; glucagon-like peptide-1 receptor; GLP-1; GLP-1R; GPCR; G protein-coupled receptor

Received
6 May 2013
Revised
10 July 2013
Accepted
19 July 2013

The incidence of type 2 diabetes in developed countries is increasing yearly with a significant negative impact on patient quality of life and an enormous burden on the healthcare system. Current biguanide and thiazolidinedione treatments for type 2 diabetes have a number of clinical limitations, the most serious long-term limitation being the eventual need for insulin replacement therapy (Table 1). Since 2007, drugs targeting the glucagon-like peptide-1 (GLP-1) receptor have been marketed for the treatment of type 2 diabetes. These drugs have enjoyed a great deal of success even though our underlying understanding of the mechanisms for their pleiotropic effects remain poorly characterized even while major pharmaceutical companies actively pursue small molecule alternatives. Coupling of the GLP-1 receptor to more than one signalling pathway (pleiotropic signalling) can result in ligand-dependent signalling bias and for a peptide receptor such as the GLP-1 receptor this can be exaggerated with the use of small molecule agonists. Better consideration of receptor signalling pleiotropy will be necessary for future drug development. This is particularly important given the recent failure of taspoglutide, the report of increased risk of pancreatitis associated with GLP-1 mimetics and the observed clinical differences between liraglutide, exenatide and the newly developed long-acting exenatide long acting release, albiglutide and dulaglutide.

LINKED ARTICLES
This article is part of a themed section on Molecular Pharmacology of GPCRs. To view the other articles in this section visit http://dx.doi.org/10.1111/bph.2014.171.issue-5

Abbreviations
ADP, adenosine diphosphate; ANS, autonomic nervous system; ATP, adenosine triphosphate; Bad, Bcl-2-associated death promoter; cAMP, cyclic adenosine monophosphate; CREB, cAMP response element binding protein; DPPIV, dipeptidyl peptidase IV; EGF-R1, epidermal growth factor receptor; Epac2, exchange protein directly activated by cAMP 2; GCGR, glucagon receptor; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide-1; GLP-1R, glucagon-like peptide-1 receptor; GLP-2, glucagon-like peptide-2; GPCR, G protein-coupled receptor; GRK, G protein-coupled receptor kinase; GSIS, glucose stimulated insulin secretion; ICV, introcerebroventricular; K_{ATP}, ATP sensitive potassium channel (potassium inwardly-rectifying channel, subfamily J); MAPK, mitogen activated protein kinase; NFAT, nuclear factor of activated T-cells; PACAP, pituitary adenylate cyclase activating polypeptide; PDX-1, pancreatic-duodenum homeobox-1; PI3K, phosphoinositide 3-kinase; PKA, protein kinase A; Rab3A, Ras-associated protein 3A; Rap1, Ras-proximate-1; Rim2, regulating synaptic membrane exocytosis 2; SNAP25, synaptosomal-associated protein 25; T2DM, type 2 diabetes mellitus; VDCC, voltage-dependant calcium channel; VIP, vasoactive intestinal peptide

The incretin effect

The observation that oral glucose administration results in significantly higher pancreatic insulin secretion compared with intravenous dosing to the same plasma concentration gave rise to the idea of an entero-insular axis that was responsible for this enhancement (Elrick *et al.*, 1964; Mcintyre *et al.*, 1964). The implication being that there was glucose sensing

Table 1

Comparison of therapies for type 2 diabetes in terms of therapeutic outcome and adverse events (TZDs = thiazolidinedione; HbA1c = glycated haemaglobin)

					GLP-1 mimetics	
	Sulfonyl-ureas	**Metformin**	**TZDs**	**DPPIV inhibitors**	**Short acting**	**Long acting**
Insulin secretion	Increased	No effect	No effect	Glucose-dependent increase	Glucose-dependent increase	Glucose-dependent increase
Beta cell glucose sensitivity	No effect	Increased	No effect	Increased	Increased	Increased
Target tissue insulin sensitivity	No effect	Increased	Increased	Unclear	Unclear	Unclear
Beta cell mass	No protection from loss	No protection from loss	No protection from loss	Unclear	Probably increased	Probably increased
HbA1c	Reduced	Reduced	Reduced	Reduced	Reduced	Reduced
Satiety	No effect	No effect	No effect	Increased	Increased	Increased
Gastric emptying	No effect	No effect	No effect	Reduced	Reduced	Small reduction
Weight	Increase	Neutral	Increase	Neutral	Sustained loss	Sustained loss
Long-term insulin dependency	Yes	Yes	Yes	Unlikely	Unlikely	Unlikely
Pancreatitis	Not reported	Not reported	Not reported	Increased risk	Increased risk	Increased risk
Adverse GIT effects	Broad spectrum GIT AEs	Mostly absent	Mostly absent	Some nausea	Some nausea	Some nausea
Hypoglycemia	At risk	Low risk	None	None	None	None

within the gastrointestinal tract or hepatic system that resulted in potentiated insulin secretion, called the incretin effect, a concept dating back to the late 19th century (reviewed in Creutzfeldt, 1979). It was shown that two gut-derived hormones gastric inhibitory polypeptide [GIP (Dupre *et al.*, 1973)] and glucagon-like peptide-1 [GLP-1 (Kreymann *et al.*, 1987)], known as incretins, were secreted in response to meal ingestion and could stimulate insulin secretion, thereby making a significant contribution to overall postprandial insulin release. Both GIP and GLP-1 have no effect on insulin secretion in the absence of elevated plasma glucose obviating the risk of hypoglycaemia (Nauck *et al.*, 1993a). However, the insulinotropic effects of GIP are lost in type 2 diabetics (T2DM) (Nauck *et al.*, 1993b), while these effects from GLP-1 are maintained, leaving GLP-1 as the primary candidate incretin for clinical use.

The insulinotropic action of GLP-1 appears to be entirely mediated by the GLP-1 receptor (GLP-1R) (Scrocchi *et al.*, 1996) [we note that the official NC-IUPHAR nomenclature (Alexander *et al.*, 2013) for this receptor is GLP-1 and have used GLP-1R to aid distinction between ligand and receptor]. Activation of the GLP-1R on pancreatic β-islets results in potentiation of glucose-dependent insulin secretion as well as improvements in beta cell function and mass in animal models (Xu *et al.*, 1999; Perfetti *et al.*, 2000; Stoffers *et al.*, 2000; Tourrel *et al.*, 2001; Farilla *et al.*, 2002; Rolin *et al.*, 2002; Wang and Brubaker, 2002; Bock *et al.*, 2003; Sturis *et al.*, 2003; Gedulin *et al.*, 2005), although some of this effect may be mediated centrally and will be discussed later. Its activation forms the basis of two classes of glucose lowering agents, incretin mimetics (i.e. GLP-1 receptor agonists) and inhibitors of dipeptidyl peptidase IV (DPPIV or CD26) (incretin enhancers), an enzyme responsible for cleavage of GLP-1 to inert metabolites.

Biology of GLP-1 synthesis, secretion and metabolism

Tissue-specific post-translational cleavage of proglucagon generates glucagon in pancreatic α-cells (Patzelt *et al.*, 1979) and GLP-1, GLP-2, oxyntomodulin and glicentin in intestinal L-cells (Orskov *et al.*, 1987; Eissele *et al.*, 1992) and the brain (Drucker and Asa, 1988; Larsen *et al.*, 1997). GLP-1(1-37) is processed in intestinal L-cells into two equipotent circulating molecular forms GLP-1(7-36) amide and GLP-1(7-37) (Holst *et al.*, 1987; Kreymann *et al.*, 1987; Mojsov *et al.*, 1987). In humans, GLP-1(7-36) amide represents the majority of circulating active GLP-1 secreted in response to nutrient ingestion (Orskov *et al.*, 1994) and will henceforth referred to simply as GLP-1. GLP-1 is secreted in a biphasic pattern with early phase beginning within 5–15 min and prolonged second phase observed from 30 to 60 min of meal ingestion (Herrmann *et al.*, 1995). GLP-1 is rapidly cleaved between positions 8 and 9 by the widely expressed serine protease DPPIV into GLP-1(9-36)amide (and GLP-1(9-37)) giving a circulation half life of 2–3 min (Mentlein *et al.*, 1993b; Deacon *et al.*, 1995; Kieffer *et al.*, 1995; Hansen *et al.*, 1999). These DPPIV-processed GLP-1 peptides have low *in vitro* affinity and activity at the classically defined human GLP-1R (Knudsen and Pridal, 1996; Montrose-Rafizadeh *et al.*, 1997a) but have

been shown to have cardioprotective and glucoregulatory actions when pharmacologically dosed (Nikolaidis *et al.*, 2005; Meier *et al.*, 2006; Elahi *et al.*, 2008) and, in model animals, may exert effects independent of GLP-1R (Ban *et al.*, 2008; 2010). The relevance of the pharmacological effects of these metabolites is unclear, particularly given they are subject to rapid renal clearance with a half-life of less than 5 min (Ruiz-Grande *et al.*, 1993; Meier *et al.*, 2004). GLP-1 is also a substrate for the metalloendopeptidase enzyme neprylisin, also known as neutral endopeptidase 24.11 (Hupe-Sodmann *et al.*, 1995). A single study in the pig demonstrated that neprylisin is responsible for degrading almost half the circulating GLP-1 (Plamboeck *et al.*, 2005), consistent with findings that the GLP-1 analogue liraglutide is also degraded by neprylsin in humans (Malm-Erjefält *et al.*, 2010). Neprylisin cleaves GLP-1 at a number of positions (Hupe-Sodmann *et al.*, 1995) and although it is theorized that these products could have mitochondrial effects (Tomas and Habener, 2010), their physiological concentrations make this unlikely.

As mentioned above, oxyntomodulin (OXM) is also a product of tissue specific cleavage of proglucagon. OXM consists of amino acids 33–69 of proglucagon and is a cleavage product of glicentin containing the full 29 amino acids of glucagon with an 8 amino-acid carboxy terminal extension (for a comprehensive review, see Holst, 2007). In both intestinal L-cells and cells of the nucleus of the solitary tract in the brain processing of proglucagon generates GLP-1, GLP-2 and glicentin which is only partly processed into OXM (Mojsov *et al.*, 1986; Orskov *et al.*, 1987; Larsen *et al.*, 1997). In the intestinal L-cells all these peptides are co-secreted in response to food intake (reviewed in Pocai, 2012). OXM is a low potency full agonist for cyclic adenosine monophosphate (cAMP) accumulation from the GLP-1R (Schepp *et al.*, 1996; Jorgensen *et al.*, 2007) and the glucagon receptor (GCGR) (Jorgensen *et al.*, 2007). It is also a full agonist for recruitment of G protein-coupled receptor kinase (GRK) 2, β-arrestin 1 and β-arrestin 2 to the GCGR; however, at the GLP-1R, OXM is only a partial agonist for these interactions (Jorgensen *et al.*, 2007). Despite this, OXM has a higher affinity for the GLP-1R than GCGR and thus, GLP-1R is proposed as the primary receptor for this peptide.

The GLP-1 receptor: structure and expression

In humans, GLP-1R is a 463 amino acid G protein-coupled receptor (GPCR) belonging to the secretin-like family (also referred to as Family B). This is a small family of only 15 GPCRs including receptors for secretin, GIP and vasoactive intestinal peptide (VIP). Structural characteristics of this receptor family include: a relatively long, extracellular N-terminal domain responsible for high affinity binding of endogenous peptide ligands; six highly conserved cysteine residues in the extracellular domain that form three conserved disulphide bridges; an amino terminal signal peptide, several N-linked glycosylation sites and of course the characteristic seven transmembrane bundle shared by all GPCRs (reviewed in Furness *et al.*, 2012).

The sites of GLP-1R expression in both model organisms and humans have been investigated using a variety of techniques of varying sensitivity and resolution. Potential roles for the receptor in physiological processes that are regulated by its ligands may be predicted by its location. In many cases there are significant shortcomings in the molecular identification of sites of expression of the GLP-1R and in these cases better molecular and functional data would be invaluable.

Expression of the GLP-1R has been demonstrated in pancreatic islets of rodents and humans (Orskov and Poulsen, 1991; Campos *et al.*, 1994; Körner *et al.*, 2007), which is consistent with the large amount of data demonstrating GLP-1 potentiation of glucose stimulated insulin secretion (GSIS). Insulin-secreting beta cells comprise 65–80% of the cells of the pancreatic islet with glucagon-secreting α-cells comprising 15–20% and somatostatin secreting δ-cells 3–10% (reviewed in In't Veld and Marichal, 2010). Based on the central location of mRNA (Bullock *et al.*, 1996; Moens *et al.*, 1996; Hörsch *et al.*, 1997) and autoradiographic GLP-1 signal (Orskov and Poulsen, 1991) and further confirmed with immunoflourescence (Tornehave *et al.*, 2008), GLP-1R is expressed on beta cells, and this expression is consistent with expression on insulinomas from rodents and humans (Göke *et al.*, 1989; Gefel *et al.*, 1990; Fehmann and Habener, 1991; Lankat-Buttgereit *et al.*, 1994). There have been reports that the GLP-1R is also expressed on both α (glucagon) and δ-(somatostatin) cells in rodents (Heller and Aponte, 1995; Heller *et al.*, 1997); however, this is not supported in immunoflourescent experiments on human islets (Tornehave *et al.*, 2008) and remains controversial. In addition to expression on islet beta cells, GLP-1R is present on the ductal exocrine cells [acinar (Xu *et al.*, 2006; Gier *et al.*, 2012)], an observation that may be important in relation to pancreatitis associated with the use of GLP-1 mimetics (see later).

GLP-1 has a number of physiological effects related to energy homeostasis and cardiovascular function that are mediated to some extent by the autonomic nervous system (ANS) and visceral afferent neurons. In both animals and humans, GLP-1 enhances satiety (Tang-Christensen *et al.*, 1996; Turton *et al.*, 1996; Flint *et al.*, 1998; Näslund *et al.*, 1998; Williams *et al.*, 2009; Kanoski *et al.*, 2011; Renner *et al.*, 2012) and inhibition of gastric emptying (Imeryüz *et al.*, 1997; Näslund *et al.*, 1998; Delgado-Aros *et al.*, 2002; Schirra *et al.*, 2002; 2006; Nagell *et al.*, 2006; Hayes *et al.*, 2008; Hellström *et al.*, 2008). Inhibition of gastric emptying is regulated primarily by the ANS and satiety is modulated by both vagal afferents and direct effects on the hypothalamus with suppression of GLP-1-dependent satiety upon vagotomy (Abbott *et al.*, 2005). There is some evidence for a GLP-1-dependent decrease in gastric acid secretion mediated by the ANS (Wettergren *et al.*, 1994; 1997; 1998). In addition to these ANS-mediated gastrointestinal effects, there are also energy homeostatic effects that appear to have some ANS contribution. In humans, depending on experimental setting, there is a GLP-1-dependent (and insulin/glucagon independent) decrease in hepatic glucose production (Prigeon *et al.*, 2003; Seghieri *et al.*, 2013), an effect that can be replicated using intracerebroventricular (ICV) administration of GLP-1 in rodents (Sandoval *et al.*, 2008; Burmeister *et al.*, 2012). In mice, ICV administration of GLP-1 stimulates thermogenesis in brown adipose (Lockie *et al.*, 2012) and

lipid deposition in white adipose is decreased (Nogueiras *et al.*, 2009). In addition, there is also evidence for GLP-1R-dependent ANS regulation of the cardiovascular system in rodents (Barragán *et al.*, 1999; Yamamoto *et al.*, 2002; Cabou *et al.*, 2008; Griffioen *et al.*, 2011), but this does not appear to be the case in humans (Bharucha *et al.*, 2008). Within the central nervous system mRNA for the GLP-1R can be detected in the thalamus, hypothalamus and brainstem in both rodents (Shughrue *et al.*, 1996; Merchenthaler *et al.*, 1999; Yamamoto *et al.*, 2003) and humans (Alvarez *et al.*, 2005) and is consistent with rodent *in situ* radioligand binding data (Göke *et al.*, 1995). These central GLP-1Rs may be stimulated by circulating GLP-1, for example in privileged areas such as the subfornical organ and the area postrema which have been shown to bind peripherally administered GLP-1 (Orskov *et al.*, 1996a), or, alternatively, may be stimulated by GLP-1 release from neurons projecting from the nucleus of the solitary tract (Jin *et al.*, 1988). There is indirect evidence in model animals for GLP-1R expression on vagal afferent nerve terminals of the hepatic portal vein (Nakabayashi *et al.*, 1996; Nishizawa *et al.*, 1996; Balkan and Li, 2000; Baumgartner *et al.*, 2010) with cell bodies in the nodose ganglion (Nakagawa *et al.*, 2004; Vahl *et al.*, 2007). Vahl *et al.* (Vahl *et al.*, 2007) use immunocytochemistry to demonstrate GLP-1R expression in hepatic portal vein nerve terminals; however, it must be noted that the antibody used is no longer available from AbCam, and the replacement does not recognize the GLP-1R (Panjwani *et al.*, 2013). Similar indirect evidence suggests GLP-1R expression on enteric neurons that communicate with the vagus nerve (Washington *et al.*, 2010).

There are conflicting reports on GLP-1/GLP-1 mimetic-dependent effects on insulin sensitivity in man with some showing no effect (Orskov *et al.*, 1996b; Vella *et al.*, 2000; 2002) and some showing enhanced sensitivity (Egan *et al.*, 2002; Zander *et al.*, 2002; Meneilly *et al.*, 2003). From the preceding discussion on the ANS, changes in liver and adipose insulin sensitivity may be influenced by the ANS (either directly or indirectly, for example via the adrenal gland, e.g. Yamamoto *et al.*, 2002; 2003); however, there may also be direct GLP-1R-mediated effects on these tissues. Whether the GLP-1R is expressed in these insulin target tissues remains contentious due to the nature of the functional experiments performed. All three tissue types are marked by unusual pharmacology in which the truncated form of exendin (exendin 9-39), which is normally considered an antagonist, as well as the pro-GLP-1 peptide GLP-1(1-36NH_2), which is generally considered low affinity (e.g. Koole *et al.*, 2010) both act as potent agonists in regulation of glucose uptake and metabolism in these cell types (Villanueva-Peñacarrillo *et al.*, 1995; Montrose-Rafizadeh *et al.*, 1997b; Wang *et al.*, 1997; Luque *et al.*, 2002; González *et al.*, 2005). This has led a number of researchers to propose that a second GLP-1 receptor exists. The characterization of exendin 9-39 and pro-GLP-1 pharmacologies as low affinity and antagonistic ligands is based on observations in transfected cell lines. The downstream pathways linking GLP-1R to glucose uptake and metabolism are not established in these cell types. It is therefore possible that these peptides have alternative, GLP-1R-dependent pharmacologies depending on cell background, particularly given the widespread heterodimerization of family B GPCRs (Harikumar *et al.*, 2008).

Beyond the above tissues, which are obvious physiological targets for GLP-1, GLP-1R expression has been reported on kidney (Korner *et al.*, 2007; Schlatter *et al.*, 2007) and lung (Kanse *et al.*, 1988; Richter *et al.*, 1990; 1993; Korner *et al.*, 2007), although expression in the human lung is restricted to small vessels (Korner *et al.*, 2007).

Molecular mechanisms underlying GLP-1R physiology

GLP-1 has multiple physiological effects that include potentiation of glucose-dependent insulin secretion, up-regulation of insulin biosynthesis, increasing beta cell mass, suppression of glucagon secretion, delaying gastric emptying, reducing appetite, improved glucose disposal and insulin sensitivity in adipose, muscle and liver as well as improving cardiovascular function and cardiovascular protective effects. In addition, GLP-1 has been reported to have anabolic effects on bone and effects on learning and memory that are beyond the scope of this review (During *et al.*, 2003; Yamada *et al.*, 2008).

The best studied molecular pathways underlying this physiology are those in the pancreas, in particular, the beta cells (Figure 1). GLP-1, acting via the GLP-1R, causes acute potentiation of GSIS, regulated primarily by signalling through the stimulatory G_α ($G_{\alpha s}$) subunit to activate adenylate cyclase and increase intracellular cAMP (Drucker *et al.*, 1987; Holst *et al.*, 1987; Mojsov *et al.*, 1987). Acute GSIS in pancreatic beta cells occurs primarily via changes in the energy balance within the cell [adenosine triphosphate (ATP)/adenosine diphosphate (ADP)], dependent on glucose catabolism. Glucose transporters are not rate limiting for glucose catabolism in beta cells, rather glucokinase provides the rate limiting step and is therefore considered the glucose sensor having high cooperativity for glucose binding and an enzymatic inflection point of ~4 mM. Increases in plasma glucose above this inflection point results in increased rates of glucose catabolism via the glycolytic and oxidative phosphorylation pathways in cytoplasm and mitochondria, respectively. The increase in ATP/ADP ratio acts on ATP sensitive potassium channels (K_{ATP}). Binding of ATP to the pore forming subunits ($K_{ir6.2}$) (Gribble *et al.*, 1998) and release of ADP from the regulatory subunits (SUR1) (Gribble *et al.*, 1997) result in channel closure leading to membrane depolarization stimulating the opening of voltage-dependent calcium channels (VDCCs) and insulin exocytosis (for reviews on GSIS see Matschinsky *et al.*, 1993; 1998; Schuit *et al.*, 2001). *In vitro* evidence from both rodent and human pancreatic islets demonstrates that GLP-1R-mediated increases in intracellular second messenger cAMP potentiates GSIS (reviewed in Gromada *et al.*, 1998). Increases in intracellular cAMP act on the K_{ATP} channel to increase its sensitivity to ATP (i.e. left shift the ATP curve). This occurs via two intermediates, protein kinase A (PKA) and the guanine nucleotide exchange factor Epac2 (Ozaki *et al.*, 2000; Kang *et al.*, 2001; Eliasson *et al.*, 2003). Both SUR1 and $K_{ir6.2}$ subunits contain consensus PKA phophorylation sites, and GLP-1R-dependent activation of PKA leads to phosphorylation of SUR1 subunits lowering their affinity for ADP (Light, 2002); however, PKA activation also changes the dynamics of exo-

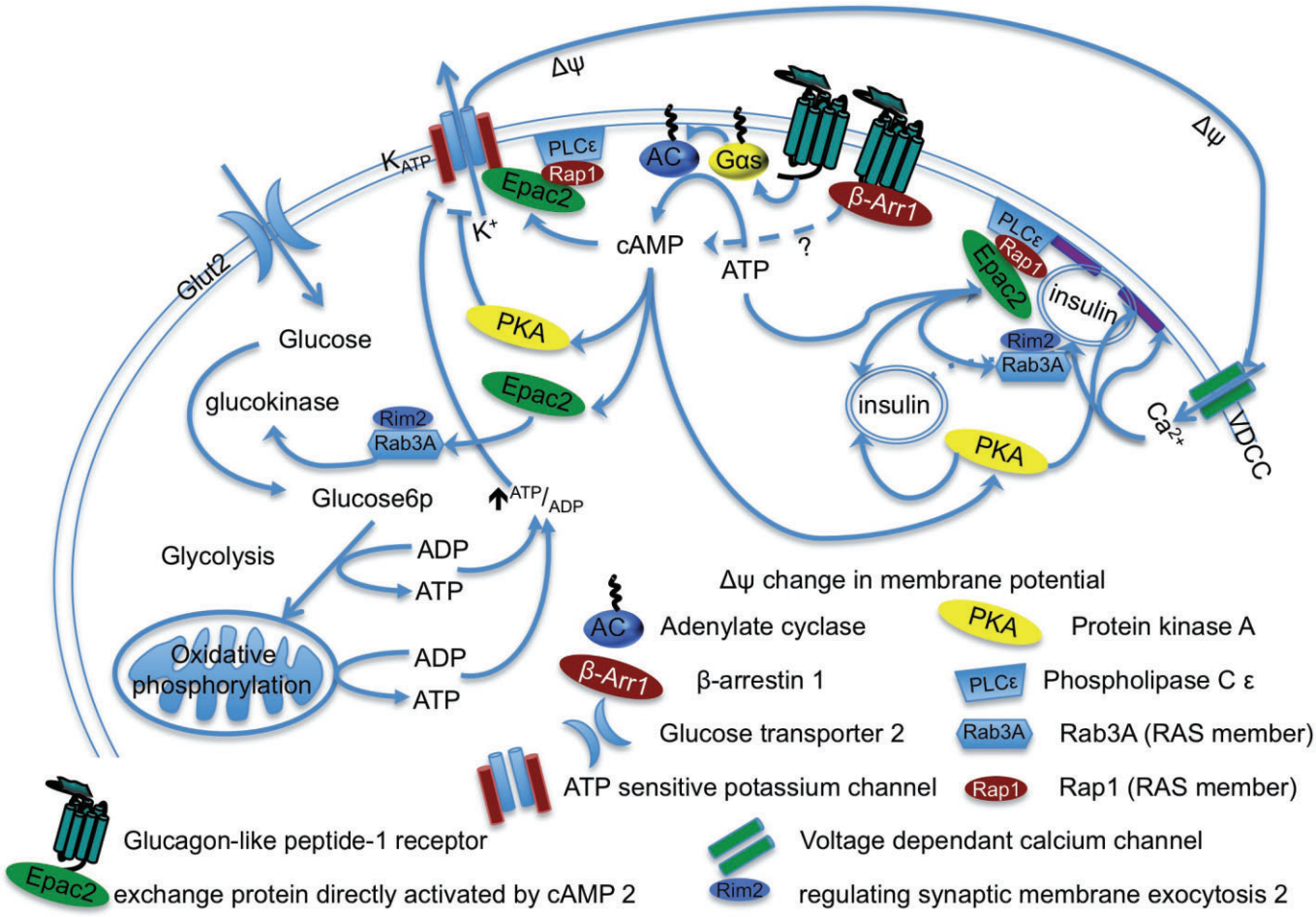

Figure 1

Cartoon depicting the major pathway by which a pancreatic beta cell secretes insulin in response to increases in plasma glucose. The various pathways that originate from GLP-1R activation and converge to potentiate glucose stimulated insulin secretion are depicted. See text for a full description.

cytosis (Eliasson *et al.*, 2003; Hatakeyama *et al.*, 2006; 2007) by phosphoylating snapin that acts to regulate vesicle assembly along with synaptosomal-associated protein 25 and Epac2 (Song *et al.*, 2011). Similarly, cAMP-dependent activation of Epac2 in beta cells increases the sensitivity of the K_{ATP} channel to ATP (Kang *et al.*, 2008) and also the dynamics of vesicle priming and fusion (Hatakeyama *et al.*, 2007; Shibasaki *et al.*, 2007; Kang *et al.*, 2008; Dzhura *et al.*, 2011) thus potentiating GSIS. Epac2 activation probably converges with PKA-dependent activation of snapin (above), but additionally the activation of the small GTPase, Rap1 (Shibasaki *et al.*, 2007) and its target phospholipase C-ε (Dzhura *et al.*, 2011) may independently potentiate vesicle priming. These GLP-1R-dependent effects to directly potentiate GSIS are $G_{\alpha s}$/cAMP dependent; however, data from the INS-1 insulinoma using supraphysiological GLP-1 stimulation in combination with β-arrestin 1 knockdown demonstrate a proportion of the cAMP/GSIS response to be β-arrestin 1 dependent (Sonoda *et al.*, 2008). Based on the observed clinical differences in effects of GLP-1 mimetics (see later), a better understanding of the requirement for this β-arrestin 1-dependent cAMP/GSIS response may be required for the development of small molecule drugs. In addition to post-glucokinase effects, GLP-1R-mediated activation of Epac2 acting in a Rim2/Rab3A-dependent manner (in rodent cell lines and islets) acutely decreases the K_m (but not Vmax) of glucokinase (Park *et al.*, 2012), thus providing an additional sensitization to GSIS, albeit at supraphysiological GLP-1 concentrations.

In addition to acute potentiation of GSIS, GLP-1R activation improves medium- and long-term insulin secretion via a number of mechanisms. In insulinoma cell lines, GLP-1R activation leads to increases in insulin expression. This effect is due to both stabilization of insulin mRNA and increases in insulin transcription (Fehmann and Habener, 1992; Wang *et al.*, 1995). GLP-1R activation in insulinoma cell lines activates cAMP response element binding protein (CREB) and NFAT in both PKA dependent and independent manners to activate insulin transcription (Skoglund *et al.*, 2000; Kemp and Habener, 2001; Chepurny, 2002; Lawrence *et al.*, 2002). The molecular pathways that connect GLP-1R to insulin transcription are however unclear, as it has been shown that GLP-1R activation can induce insulin expression indepen-

dent of cAMP, PKA and Epac2. Insulin secretion is also dependent on beta cell mass, and GLP-1R activation is associated with trophic and protective effects including proliferation, anti-apoptosis and stimulation of islet neogenesis. In insulinoma cell lines activation of GLP-1R generates cell autonomous signalling via β-arrestin 1 to inhibit the activity of pro-apoptotic protein Bad (Quoyer *et al.*, 2010) and paracrine pro-proliferative effects via post-transcriptional up-regulation of epidermal growth factor receptor (EGF-R1) (Buteau *et al.*, 2003; Cornu *et al.*, 2010). Isolated human islets are also protected from apoptosis by GLP-1 (Farilla *et al.*, 2003); however, this may be compensatory due to loss of tonic stimulation of cAMP (compare Xie *et al.*, 2007 and Preitner *et al.*, 2004). In glucose intolerant rats, GLP-1 increases beta cell mass correlating with induction of expression of the beta cell transcriptional regulator pancreatic-duodenum homeobox-1 (PDX-1) (Perfetti *et al.*, 2000). GLP-1R-dependent activation of PDX-1 is also seen in model insulinomas and is dependent on a cAMP/PKA pathway (Wang *et al.*, 2001), and induction of PDX-1 expression is likely to contribute directly to islet neogenesis (Hui *et al.*, 2001; Bulotta *et al.*, 2002). In addition to these direct effects, vagal innervation of the pancreas is well documented to regulate beta cell mass (e.g. Lausier *et al.*, 2010; Llewellyn-Smith and Verberne, 2013), and GLP-1R activation stimulates pancreas projecting vagal neurons (Wan *et al.*, 2007a,b). Beyond GLP-1R-mediated effects on the endocrine pancreas, rodent models show significant adverse effects in exocrine pancreas. In a 10 weeks trial in rats, exendin treatment resulted in significant acinar cell death and inflammation (Nachnani *et al.*, 2010) and in a mouse model of pancreatic intraepithelia neoplasia, exendin treatment significantly accelerated metaplasia and lesion formation (Gier *et al.*, 2012). While there is no mechanistic data to explain these exocrine pancreatic effects, recent meta analysis on the incidence of pancreatitis in T2DM patients shows a doubling in relative risk for those using GLP-1 therapies (Singh *et al.*, 2013). The pleiotropic effects on beta cell function and mass are clinically very important as preservation of pancreatic function is one of the key advantages to GLP-1-based therapies. It is also imperative that the nature of GLP-1 pathways that form the molecular basis for pancreatitis be established. These pathways may be via changes in pancreatic morphology due to accelerated beta cell neogenesis or via direct effects on acinar cells that express GLP-1R.

The ability of GLP-1 to suppress glucagon secretion from pancreatic α-cells is regarded as important for its glucoregulatory effects. Under conditions of hyperinsulinaemic-euglycaemic clamp, GLP-1 does not inhibit glucagon secretion in humans (Meneilly *et al.*, 2003). This suggests that GLP-1-dependent regulation of glucagon secretion is not mediated by direct effects on pancreatic α-cells but rather is mediated by a combination of pancreatic paracrine (Franklin *et al.*, 2005) and ANS signalling (Llewellyn-Smith and Verberne, 2013).

The ANS effects of GLP-1 are critical for the success of GLP-1 mimetics as treatments for T2DM. The effect of GLP-1 mimetics on postprandial glucose excursion is largely via slowing in gastric emptying (Meier *et al.*, 2005; Linnebjerg *et al.*, 2008); however, this effect is subject to tachyphylaxis (Nauck *et al.*, 2011) and as a result, long-acting GLP-1 analogues are less efficacious in controlling postprandial glucose excursion (Degn *et al.*, 2004; Buse *et al.*, 2009). GLP-1-dependent decreases in gastric emptying appear to be mediated by the ANS (Nagell *et al.*, 2006; Holmes *et al.*, 2009) suggesting that the sites of GLP-1R expression responsible for gastric emptying are sites in which GLP-1R is subject to agonist mediated desensitization. In contrast, similar weight loss is observed in T2DM patients receiving both long- and short-acting GLP-1 mimetics (Drucker *et al.*, 2008; Buse *et al.*, 2009), consistent with the gastric motility independent decrease in food intake seen in ICV-treated rats (Turton *et al.*, 1996) that is apparently not subject to GLP-1R desensitization. It is therefore likely that there are some sites in these pathways where GLP-1R is desensitized and some are not and this is likely to be an important consideration for the design of small molecule GLP-1R agonists. There is one site in which GLP-1R stimulation has been shown to result in neuronal depolarization (e.g. Wan *et al.*, 2007a,b; Holmes *et al.*, 2009); however, there is no data that address the pathways immediately downstream of the GLP-1R that cause depolarization, and there is no data that allow this site to be extrapolated to sites important for gastric emptying and satiety. An understanding of the subsets of neurons activated and the downstream pathways required for the therapeutic effects is important as there are clear differences in the anorectic effects of different GLP-1R agonists. In rodents, GLP-1 and exendin drive similar reductions in food intake when administered peripherally, but exendin is far more potent when administered ICV (Barrera *et al.*, 2009); in clinical trials, high molecular weight GLP-1 mimetics (e.g. Albiglutide and Dulaglutide) fail to provide weight loss benefits seen with peptide mimetics (Rosenstock *et al.*, 2009; Grunberger *et al.*, 2012) suggesting differential access by these ligands to central GLP-1R.

As part of its overall glucoregulatory role, GLP-1 can act to inhibit endogenous glucose production in the liver both acutely (Meneilly *et al.*, 2003; Prigeon *et al.*, 2003) and over the long term (Egan *et al.*, 2002; Zander *et al.*, 2002). Although GLP-1 binding has been demonstrated on rat hepatocyte membranes (Villanueva-Peñacarrillo *et al.*, 1995), there is no convincing evidence that GLP-1 agonists exert a direct effect on liver metabolism, rather GLP-1 effects on hepatic function, where they occur, are likely to be centrally mediated (although this may be indirect). In human adipocytes, there is one report showing GLP-1 potentiation of glucose uptake via phosphoinositide 3-kinase (PI3K) and mitogen-activated protein kinase pathways (Sancho *et al.*, 2007). In this report, exendin 9-39 also stimulates PI3K activity that contrasts to the traditional view of this ligand as an antagonist. In addition, the data from the mouse suggest that, rather than stimulating glucose uptake and therefore lipogenesis, GLP-1 acts to increase lipolysis. Given one of the useful clinical outcomes of GLP-1 mimetics is sustained weight loss, a better understanding of the effects on adipose physiology is required.

Our understanding of the underlying molecular basis of GLP-1's pleiotropic effects would benefit greatly from high quality antibodies directed against GLP-1R and a GLP-1R reporter mouse so the precise location of receptors in different tissues could be fully characterized. Better characterization of pathways leading to translational and post-translational control of gene expression by GLP-1R activation

is required. Additionally, comprehensive studies that provide pharmacological or biochemical characterization of GLP-1R on tissues such as liver and adipose would greatly enhance our understanding of the underlying physiology.

Challenges for incretin therapies for T2DM

Currently there are no therapeutically approved small molecule GLP-1R agonists. As discussed above, the therapeutic usefulness of GLP-1 (peptide) is limited by its metabolic instability. Thus, therapeutic strategies for increasing active GLP-1 concentrations include DPP4 resistant GLP-1 mimetics such as exenatide and liraglutide or DPP4 inhibitors such as vildagliptin and sitagliptin that are approved as monotherapies and/or adjuvant drugs with oral antidiabetic compounds, depending on the country of approval.

Approved DPPIV inhibitors (known as gliptins) show very good selectivity for DPPIV over the structurally related enzymes DPPVIII and DPPIX (Brandt *et al.*, 2005; Kim *et al.*, 2005; Feng *et al.*, 2007; Wang *et al.*, 2012). DPPIV inhibitors produce modest changes in circulating levels of active GLP-1 (GLP-1(7-36)NH_2) levels ranging from twofold to fourfold depending on the study (Ahrén *et al.*, 2004; Herman *et al.*, 2005; Dai *et al.*, 2008; Henry *et al.*, 2011). For healthy individuals, this corresponds to an increase in fasted GLP-1 to ~3 pM and postprandial peak of ~18 pM, compared with T2DM patients where reduced GLP-1 secretion is observed and the plasma concentrations are roughly half. Although the available data indicate that DPPIV inhibition is a promising treatment for type 2 diabetes, gliptins are clinically less effective than GLP-1 mimetics (reviewed in Brown and Evans, 2012; Reid, 2012; Scheen, 2012). There are also concerns that prolonged inhibition of DPPIV activity could lead to adverse side effects. Substrate cleavage by DPPIV occurs at penultimate L-proline or L-alanine residues (Kenny *et al.*, 1976). Some known and putative *in vivo* substrates of DPPIV include substance P (Heymann and Mentlein, 1978; Kato *et al.*, 1978), neuropeptide Y and peptide YY (Mentlein *et al.*, 1993a), endomorphin-1 (Bird *et al.*, 2001), pituitary adenylate cyclase activating peptide 38 (PACAP 38) (Lambeir *et al.*, 2001); GLP-1 and GIP (Mentlein *et al.*, 1993b), GLP-2 (Drucker *et al.*, 1997) and various chemokines such as CCL5 (regulated on activation, normal T-cell expressed and secreted, RANTES) and CCL11 (eotaxin) (Oravecz *et al.*, 1997), CCL22 (macrophage derived chemokine, MDC) (Proost *et al.*, 1999), CCL3L1 (LD78β) (Proost *et al.*, 2000) and CXCL12 (stromal-cell-derived factor 1, SDF-1) (Proost *et al.*, 1998). Thus, inhibition of DPPIV could potentially extend the circulating half-lives of these biologically active peptides which might conceivably affect/modulate vasoreactivity, nociception, energy homeostasis (food intake, lipid metabolism, thermogenesis and glucose control), proliferation, angiogenesis, immune response, behavioural stress response, gastrointestinal motility and growth.

Currently there are a number of GLP-1 analogues that have been approved or are in clinical trials for treatment of T2DM (Figure 2). The main drawback of all GLP-1 analogues is the need for parenteral administration. There has therefore been substantial effort to extend the half-life of these mimetics. Two synthetic analogues of exendin-4, exenatide and lixisenatide, were isolated from the saliva of the Gila monster (*Heloderma suspectum*). These two analogues are naturally resistant to DPPIV; exenatide and lixisenatide, which has a 6

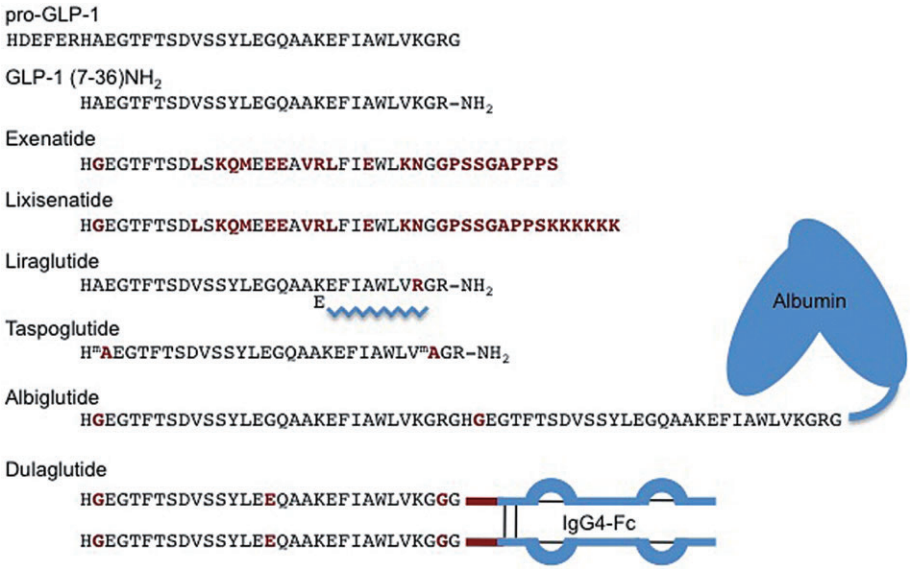

Figure 2

A schematic of the various GLP-1 mimetics discussed in the text. Differences in sequence with respect to native GLP-1 are shown in red. Albiglutide is a genetic fusion of a GLP-1 concatamer to human albumin (blue), and Dulaglutide is a genetic fusion of GLP-1 via a linker (red) to the Fc domain of human IgG4 (blue).
Figure 2 was amended after first publication to correct the Dulaglutide sequence.

amino acid polylysine extension at the C-terminus, have circulating half lives of around 4 h but have been formulated for once a day injection. Liraglutide is based on the mammalian GLP-1 sequence with a glutamate and 16 carbon fatty acid conjugated to the ε-amino group of lysine26 and a substitution of lysine34 with arginine. This modification increases albumin binding to 99%, thus protecting liraglutide from DPPIV degradation and giving it an 11–13 h half life. Taspoglutide is also based on mammalian GLP-1 with substitution of Ala8 and Ala35 with methylated derivatives, thus protecting the peptide from DPPIV and giving a half-life of 10 h. Albiglutide uses two molecules of gly8 substituted GLP-1 sequences covalently coupled to human albumin and has a half life of 6–8 days. Dulaglutide uses two molecules of a GLP-1 sequence substituted at position 8 with glycine, 22 with glutamic acid and 26 with glycine fused via a linker to human IgG4-Fc domain and has a half life of 4 days (Glaesner *et al.*, 2010).* Lastly exenatide-LAR is a microsphere formulated extended release formulation of exendin that is suitable for once a week injection. These GLP-1 analogues are very selective for GLP-1R acitivation and do not suffer from the potential off target affects of DPPIV inhibitors. Clinically, all GLP-1 mimetics are better at lowering fasting and postprandial plasma glucose, glycosylated haemaglobin levels and weight compared with DPPIV inhibitors (Table 1; Arnolds *et al.*, 2010; Pratley *et al.*, 2010; 2011; Berg *et al.*, 2011). In spite of these positive attributes, there are differences in the clinical outcomes from trials using these different analogues that point to differential GLP-1R activation or signal bias by these ligands as well as safety concerns. In spite of the relatively minor changes in sequence for taspoglutide compared with mammalian GLP-1, this drug has now been withdrawn from clinical trials due to unacceptably high incidence of nausea and vomiting as well as systemic and injection site allergic reactions (Rosenstock *et al.*, 2013). It is unclear why this mimetic displays such a different emetic effect compared with liraglutide that has similar pharmacokinetics, however we would speculate that this may be due to differences in the mechanism of GLP-1R activation or access. Additionally, GLP-1 desensitization may play an important role in the tolerability of GLP-1 mimetics. All GLP-1 mimetics have nausea and vomiting as side effects; however, these side effects generally subside over time. It is therefore possible that the clinical difference seen with taspoglutide is due to failure of reeptor desensitization. Although exenatide-LAR, albiglutide and dulaglutide offer the benefit of once weekly injection, these long-acting analogues do not offer the same level of weight reduction seen with the short-acting analogues and this appears to be due to their reduced ability to promote satiety. The large conjugates, albiglutide and dulaglutide, also appear to be poor potentiators of GLP-1R anorectic effects, and this is probably due to the inability of these large molecules to access areas of the central nervous system (CNS) important for this effect. Alternatively, all long-acting mimetics may be less effective at promoting satiety due to them causing receptor desensitization in parts of the ANS responsible for satiety. If this is the case, a thorough understanding of the different neuronal types and the mechanisms for GLP-1R desensitization in these neurons will be necessary. Lastly, although evidence has not been published for albiglutide and dulaglutide, both DPPIV inhibitors and GLP-1 mimetics double the risk of pancreatitis, a condition associated with serious morbidity. This effect is almost certainly due to GLP-1R activation on acinar cells of the exocrine pancreas leading to their apoptosis and metaplasia and causing associated inflammation. In pancreatic beta cells, GLP-1R activation has anti-apoptotic and pro-proliferative effects, whereas in acinar ductal cells, it appears to be pro-apoptotic and drive metaplastic differentiation. In a subset of neurons responsible for GLP-1-mediated gastric emptying, sustained activation does not appear to be susceptible to tachyphylaxis whereas the neurons responsible for appetite suppression are. It may well be possible to generate small molecule ligands for the GLP-1R that display a suitable bias profile so as to bypass the pancreatic intraepithelial neoplasty while improving the weight loss profile through lack of receptor desensitization in the CNS. For these challenges to be met, a better understanding or key aspects of GLP-1R physiology and underlying molecular signalling is required.

*This sentence was amended after first publication to correct the substitution positions in Dulaglutide.

Acknowledgements

This work was funded by Program Grant 519461 and Project Grant 436780 from the National Health and Medical Research Council (NHMRC) of Australia. We would like to thank Dane D. Jensen for helpful discussions and John B. Furness for critical evaluation of the manuscript.

Conflict of interest

None declared.

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.

Ahrén B, Landin-Olsson M, Jansson P-A, Svensson M, Holmes D, Schweizer A (2004). Inhibition of dipeptidyl peptidase-4 reduces glycemia, sustains insulin levels, and reduces glucagon levels in type 2 diabetes. J Clin Endocrinol Metab 89: 2078–2084.

Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Catterall WA, Spedding M, Peters JA, Harmar AJ and CGTP Collaborators (2013). The Concise Guide to PHARMACOLOGY 2013/14: G Protein-Coupled Receptors. Br J Pharmacol 170: 1459–1581.

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.

Arnolds S, Dellweg S, Clair J, Dain M-P, Nauck MA, Rave K *et al.* (2010). Further improvement in postprandial glucose control with

addition of exenatide or sitagliptin to combination therapy with insulin glargine and metformin: a proof-of-concept study. Diabetes Care 33: 1509–1515.

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.

Ban K, Noyan-Ashraf MH, Hoefer J, Bolz S-S, Drucker DJ, Husain M (2008). Cardioprotective and vasodilatory actions of glucagon-like peptide 1 receptor are mediated through both glucagon-like peptide 1 receptor-dependent and -independent pathways. Circulation 117: 2340–2350.

Ban K, Kim KH, Cho CK, Sauve M, Diamandis EP, Backx PH *et al.* (2010). Glucagon-like peptide (GLP)-1(9-36)amide-mediated cytoprotection is blocked by exendin(9-39) yet does not require the known GLP-1 receptor. Endocrinology 151: 1520–1531.

Barragán JM, Eng J, Rodríguez R, Blázquez E (1999). Neural contribution to the effect of glucagon-like peptide-1-(7-36) amide on arterial blood pressure in rats. Am J Physiol 277: E784–E791.

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.

Baumgartner I, Pacheco-López G, Rüttimann EB, Arnold M, Asarian L, Langhans W *et al.* (2010). Hepatic-portal vein infusions of glucagon-like peptide-1 reduce meal size and increase c-Fos expression in the nucleus tractus solitarii, area postrema and central nucleus of the amygdala in rats. J Neuroendocrinol 22: 557–563.

Berg JK, Shenouda SK, Heilmann CR, Gray AL, Holcombe JH (2011). Effects of exenatide twice daily versus sitagliptin on 24-h glucose, glucoregulatory and hormonal measures: a randomized, double-blind, crossover study. Diabetes Obes Metab 13: 982–989.

Bharucha AE, Charkoudian N, Andrews CN, Camilleri M, Sletten D, Zinsmeister AR *et al.* (2008). Effects of glucagon-like peptide-1, yohimbine, and nitrergic modulation on sympathetic and parasympathetic activity in humans. Am J Physiol Regul Integr Comp Physiol 295: R874–R880.

Bird AP, Faltinek JR, Shojaei AH (2001). Transbuccal peptide delivery: stability and in vitro permeation studies on endomorphin-1. J Control Release 73: 31–36.

Bock T, Pakkenberg B, Buschard K (2003). The endocrine pancreas in non-diabetic rats after short-term and long-term treatment with the long-acting GLP-1 derivative NN2211. Apmis 111: 1117–1124.

Brandt I, Joossens J, Chen X, Maes M-B, Scharpé S, De Meester I *et al.* (2005). Inhibition of dipeptidyl-peptidase IV catalyzed peptide truncation by Vildagliptin ((2S)-{[(3-hydroxyadamantan-1-yl)amino] acetyl}-pyrrolidine-2-carbonitrile). Biochem Pharmacol 70: 134–143.

Brown DX, Evans M (2012). Choosing between GLP-1 receptor agonists and DPP-4 inhibitors: a pharmacological perspective. J Nutr Metab 2012: 1–10.

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.

Bulotta A, Hui H, Anastasi E, Bertolotto C, Boros LG, Di Mario U *et al.* (2002). Cultured pancreatic ductal cells undergo cell cycle re-distribution and beta-cell-like differentiation in response to glucagon-like peptide-1. J Mol Endocrinol 29: 347–360.

Burmeister MA, Ferre T, Ayala JE, King EM, Holt RM, Ayala JE (2012). Acute activation of central GLP-1 receptors enhances hepatic insulin action and insulin secretion in high-fat-fed, insulin resistant mice. Am J Physiol Endocrinol Metab 302: E334–E343.

Buse JB, Rosenstock J, Sesti G, Schmidt WE, Montanya E, Brett JH *et al.* (2009). Liraglutide once a day versus exenatide twice a day for type 2 diabetes: a 26-week randomised, parallel-group, multinational, open-label trial (LEAD-6). Lancet 374: 39–47.

Buteau J, Foisy S, Joly E, Prentki M (2003). Glucagon-like peptide 1 induces pancreatic β-cell proliferation via transactivation of the epidermal growth factor receptor. Diabetes 52: 124–132.

Cabou C, Campistron G, Marsollier N, Leloup C, Cruciani-Guglielmacci C, Pénicaud L *et al.* (2008). Brain glucagon-like peptide-1 regulates arterial blood flow, heart rate, and insulin sensitivity. Diabetes 57: 2577–2587.

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.

Chepurny OG (2002). Exendin-4 as a stimulator of rat insulin I gene promoter activity via bZIP/CRE interactions sensitive to serine/threonine protein kinase inhibitor Ro 31-8220. Endocrinology 143: 2303–2313.

Cornu M, Modi H, Kawamori D, Kulkarni RN, Joffraud M, Thorens B (2010). Glucagon-like peptide-1 increases beta-cell glucose competence and proliferation by translational induction of insulin-like growth factor-1 receptor expression. J Biol Chem 285: 10538–10545.

Creutzfeldt W (1979). The incretin concept today. Diabetologia 16: 75–85.

Dai H, Gustavson SM, Preston GM, Eskra JD, Calle R, Hirshberg B (2008). Non-linear increase in GLP-1 levels in response to DPP-IV inhibition in healthy adult subjects. Diabetes Obes Metab 10: 506–513.

Deacon CF, Johnsen AH, Holst JJ (1995). Degradation of glucagon-like peptide-1 by human plasma in vitro yields an N-terminally truncated peptide that is a major endogenous metabolite in vivo. J Clin Endocrinol Metab 80: 952–957.

Degn KB, Juhl CB, Sturis J, Jakobsen G, Brock B, Chandramouli V *et al.* (2004). One week's treatment with the long-acting glucagon-like peptide 1 derivative liraglutide (NN2211) markedly improves 24-h glycemia and alpha- and beta-cell function and reduces endogenous glucose release in patients with type 2 diabetes. Diabetes 53: 1187–1194.

Delgado-Aros S, Kim D-Y, Burton DD, Thomforde GM, Stephens D, Brinkmann BH *et al.* (2002). Effect of GLP-1 on gastric volume, emptying, maximum volume ingested, and postprandial symptoms in humans. Am J Physiol Gastrointest Liver Physiol 282: G424–G431.

Drucker DJ, Asa S (1988). Glucagon gene expression in vertebrate brain. J Biol Chem 263: 13475–13478.

Drucker DJ, Philippe J, Mojsov S, Chick WL, Habener JF (1987). Glucagon-like peptide I stimulates insulin gene expression and increases cyclic AMP levels in a rat islet cell line. Proc Natl Acad Sci U S A 84: 3434–3438.

Drucker DJ, Shi Q, Crivici A, Sumner-Smith M, Tavares W, Hill M *et al.* (1997). Regulation of the biological activity of glucagon-like peptide 2 in vivo by dipeptidyl peptidase IV. Nat Biotechnol 15: 673–677.

Drucker DJ, Buse JB, Taylor K, Kendall DM, Trautmann M, Zhuang D *et al.* (2008). Exenatide once weekly versus twice daily for the treatment of type 2 diabetes: a randomised, open-label, non-inferiority study. Lancet 372: 1240–1250.

Dupre J, Ross SA, Watson D, Brown JC (1973). Stimulation of insulin secretion by gastric inhibitory polypeptide in man. J Clin Endocrinol Metab 37: 826–828.

During MJ, Cao L, Zuzga DS, Francis JS, Fitzsimons HL, Jiao X *et al.* (2003). Glucagon-like peptide-1 receptor is involved in learning and neuroprotection. Nat Med 9: 1173–1179.

Dzhura I, Chepurny OG, Leech CA, Roe MW, Dzhura E, Xu X *et al.* (2011). Phospholipase C-ε links Epac2 activation to the potentiation of glucose-stimulated insulin secretion from mouse islets of Langerhans. Islets 3: 121–128.

Egan JM, Meneilly GS, Habener JF, Elahi D (2002). Glucagon-like peptide-1 augments insulin-mediated glucose uptake in the obese state. J Clin Endocrinol Metab 87: 3768–3773.

Eissele R, Göke R, Willemer S, Harthus HP, Vermeer H, Arnold R *et al.* (1992). Glucagon-like peptide-1 cells in the gastrointestinal tract and pancreas of rat, pig and man. Eur J Clin Invest 22: 283–291.

Elahi D, Egan JM, Shannon RP, Meneilly GS, Khatri A, Habener JF *et al.* (2008). GLP-1 (9-36) amide, cleavage product of GLP-1 (7-36) amide, is a glucoregulatory peptide. Obesity (Silver Spring) 16: 1501–1509.

Eliasson L, Ma X, Renström E, Barg S, Berggren P-O, Galvanovskis J *et al.* (2003). SUR1 regulates PKA-independent cAMP-induced granule priming in mouse pancreatic B-cells. J Gen Physiol 121: 181–197.

Elrick H, Stimmler L, Hlad CJ, Arai Y (1964). Plasma insulin response to oral and intravenous glucose administration. J Clin Endocrinol Metab 24: 1076–1082.

Farilla L, Hui H, Bertolotto C, Kang E, Bulotta A, Di Mario U *et al.* (2002). Glucagon-like peptide-1 promotes islet cell growth and inhibits apoptosis in Zucker diabetic rats. Endocrinology 143: 4397.

Farilla L, Bulotta A, Hirshberg B, Li Calzi S, Khoury N, Noushmehr H *et al.* (2003). Glucagon-like peptide 1 inhibits cell apoptosis and improves glucose responsiveness of freshly isolated human islets. Endocrinology 144: 5149–5158.

Fehmann HC, Habener JF (1991). Homologous desensitization of the insulinotropic glucagon-like peptide-I (7-37) receptor on insulinoma (HIT-T15) cells. Endocrinology 128: 2880–2888.

Fehmann HC, Habener JF (1992). Insulinotropic hormone glucagon-like peptide-I(7-37) stimulation of proinsulin gene expression and proinsulin biosynthesis in insulinoma beta TC-1 cells. Endocrinology 130: 159–166.

Feng J, Zhang Z, Wallace MB, Stafford JA, Kaldor SW, Kassel DB *et al.* (2007). Discovery of alogliptin: a potent, selective, bioavailable, and efficacious inhibitor of dipeptidyl peptidase IV. J Med Chem 50: 2297–2300.

Flint A, Raben A, Astrup A, Holst JJ (1998). Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. J Clin Invest 101: 515–520.

Franklin I, Gromada J, Gjinovci A, Theander S, Wollheim CB (2005). β-cell secretory products activate α-cell ATP-dependent potassium channels to inhibit glucagon release. Diabetes 54: 1808–1815.

Furness SG, Wootten D, Christopoulos A, Sexton PM (2012). Consequences of splice variation on secretin family G protein-coupled receptor function. Br J Pharmacol 166: 98–109.

Gedulin BR, Nikoulina SE, Smith PA, Gedulin G, Nielsen LL, Baron AD *et al.* (2005). Exenatide (exendin-4) improves insulin sensitivity and {beta}-cell mass in insulin-resistant obese fa/fa Zucker rats independent of glycemia and body weight. Endocrinology 146: 2069–2076.

Gefel D, Hendrick GK, Mojsov S, Habener J, Weir GC (1990). Glucagon-like peptide-I analogs: effects on insulin secretion and adenosine 3′,5′-monophosphate formation. Endocrinology 126: 2164–2168.

Gier B, Matveyenko AV, Kirakossian D, Dawson D, Dry SM, Butler PC (2012). Chronic GLP-1 receptor activation by exendin-4 induces expansion of pancreatic duct glands in rats and accelerates formation of dysplastic lesions and chronic pancreatitis in the Kras(G12D) mouse model. Diabetes 61: 1250–1262.

Glaesner W, Vick AM, Millican R, Ellis B, Tschang SH, Tian Y, *et al.* (2010). Engineering and characterization of the long-acting glucagon-like peptide-1 analogue LY2189265, an Fc fusion protein. Diabetes Metab Res Rev 26: 287–296.

Göke R, Trautmann ME, Haus E, Richter G, Fehmann HC, Arnold R, Göke B (1989). Signal transmission after GLP-1(7-36)amide binding in RINm5F cells. Am J Physiol 257: G397–G401.

Göke R, Larsen PJ, Mikkelsen JD, Sheikh SP (1995). Distribution of GLP-1 binding sites in the rat brain: evidence that exendin-4 is a ligand of brain GLP-1 binding sites. Eur J Neurosci 7: 2294–2300.

González N, Acitores A, Sancho V, Valverde I, Villanueva-Peñacarrillo ML (2005). Effect of GLP-1 on glucose transport and its cell signalling in human myocytes. Regul Pept 126: 203–211.

Gribble FM, Tucker SJ, Ashcroft FM (1997). The essential role of the Walker A motifs of SUR1 in K-ATP channel activation by Mg-ADP and diazoxide. EMBO J 16: 1145–1152.

Gribble FM, Tucker SJ, Haug T, Ashcroft FM (1998). MgATP activates the beta cell KATP channel by interaction with its SUR1 subunit. Proc Natl Acad Sci U S A 95: 7185–7190.

Griffioen KJ, Wan R, Okun E, Wang X, Lovett-Barr MR, Li Y *et al.* (2011). GLP-1 receptor stimulation depresses heart rate variability and inhibits neurotransmission to cardiac vagal neurons. Cardiovasc Res 89: 72–78.

Gromada J, Holst JJ, Rorsman P (1998). Cellular regulation of islet hormone secretion by the incretin hormone glucagon-like peptide 1. Pflugers Arch 435: 583–594.

Grunberger G, Chang A, Garcia Soria G, Botros FT, Bsharat R, Milicevic Z (2012). Monotherapy with the once-weekly GLP-1 analogue dulaglutide for 12 weeks in patients with type 2 diabetes: dose-dependent effects on glycaemic control in a randomized, double-blind, placebo-controlled study. Diabet Med 29: 1260–1267.

Hansen L, Deacon CF, Orskov C, Holst JJ (1999). Glucagon-like peptide-1-(7-36)amide is transformed to glucagon-like peptide-1-(9-36)amide by dipeptidyl peptidase IV in the capillaries supplying the L cells of the porcine intestine. Endocrinology 140: 5356–5363.

Harikumar KG, Morfis MM, Sexton PM, Miller LJ (2008). Pattern of intra-family hetero-oligomerization involving the G-protein-coupled secretin receptor. J Mol Neurosci 36: 279–285.

Hatakeyama H, Kishimoto T, Nemoto T, Kasai H, Takahashi N (2006). Rapid glucose sensing by protein kinase A for insulin exocytosis in mouse pancreatic islets. J Physiol (Lond) 570: 271–282.

Hatakeyama H, Takahashi N, Kishimoto T, Nemoto T, Kasai H (2007). Two cAMP-dependent pathways differentially regulate exocytosis of large dense-core and small vesicles in mouse beta-cells. J Physiol (Lond) 582: 1087–1098.

Hayes MR, Skibicka KP, Grill HJ (2008). Caudal brainstem processing is sufficient for behavioral, sympathetic, and parasympathetic responses driven by peripheral and hindbrain glucagon-like-peptide-1 receptor stimulation. Endocrinology 149: 4059–4068.

Heller RS, Aponte GW (1995). Intra-islet regulation of hormone secretion by glucagon-like peptide-1-(7–36) amide. Am J Physiol 269: G852–G860.

Heller RS, Kieffer TJ, Habener JF (1997). Insulinotropic glucagon-like peptide I receptor expression in glucagon-producing alpha-cells of the rat endocrine pancreas. Diabetes 46: 785–791.

Hellström PM, Näslund E, Edholm T, Schmidt PT, Kristensen J, Theodorsson E *et al*. (2008). GLP-1 suppresses gastrointestinal motility and inhibits the migrating motor complex in healthy subjects and patients with irritable bowel syndrome. Neurogastroenterol Motil 20: 649–659.

Henry RR, Smith SR, Schwartz SL, Mudaliar SR, Deacon CF, Holst JJ *et al*. (2011). Effects of saxagliptin on β-cell stimulation and insulin secretion in patients with type 2 diabetes. Diabetes Obes Metab 13: 850–858.

Herman GA, Stevens C, Van Dyck K, Bergman A, Yi B, De Smet M *et al*. (2005). Pharmacokinetics and pharmacodynamics of sitagliptin, an inhibitor of dipeptidyl peptidase IV, in healthy subjects: results from two randomized, double-blind, placebo-controlled studies with single oral doses. Clin Pharmacol Ther 78: 675–688.

Herrmann C, Göke R, Richter G, Fehmann HC, Arnold R, Göke B (1995). Glucagon-like peptide-1 and glucose-dependent insulin-releasing polypeptide plasma levels in response to nutrients. Digestion 56: 117–126.

Heymann E, Mentlein R (1978). Liver dipeptidyl aminopeptidase IV hydrolyzes substance P. FEBS Lett 91: 360–364.

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 (Lond) 587: 4749–4759.

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

Holst JJ, Orskov C, Vagn Nielsen O, Schwartz TW (1987). Truncated glucagon-like peptide I, an insulin-releasing hormone from the distal gut. FEBS Lett 211: 169–174.

Hörsch D, Göke R, Eissele R, Michel B, Göke B (1997). Reciprocal cellular distribution of glucagon-like peptide-1 (GLP-1) immunoreactivity and GLP-1 receptor mRNA in pancreatic islets of rat. Pancreas 14: 290–294.

Hui H, Wright C, Perfetti R (2001). Glucagon-like peptide 1 induces differentiation of islet duodenal homeobox-1-positive pancreatic ductal cells into insulin-secreting cells. Diabetes 50: 785–796.

Hupe-Sodmann K, McGregor GP, Bridenbaugh R, Göke R, Göke B, Thole H *et al*. (1995). Characterisation of the processing by human neutral endopeptidase 24.11 of GLP-1(7-36) amide and comparison of the substrate specificity of the enzyme for other glucagon-like peptides. Regul Pept 58: 149–156.

Imeryüz N, Yeğen BÇ, Bozkurt A, Coşkun T, Villanueva-Peñacarrillo ML, Ulusoy NB (1997). Glucagon-like peptide-1 inhibits gastric emptying via vagal afferent-mediated central mechanisms. Am J Physiol 273: G920–G927.

In't Veld P, Marichal M (2010). Microscopic anatomy of the human islet of Langerhans. Adv Exp Med Biol 654: 1–19.

Jin SL, Han VK, Simmons JG, Towle AC, Lauder JM, Lund PK (1988). Distribution of glucagonlike peptide I (GLP-I), glucagon, and glicentin in the rat brain: an immunocytochemical study. J Comp Neurol 271: 519–532.

Jorgensen R, Kubale V, Vrecl M, Schwartz TW, Elling CE (2007). Oxyntomodulin differentially affects glucagon-like peptide-1 receptor beta-arrestin recruitment and signaling through Galpha(s). J Pharmacol Exp Ther 322: 148–154.

Kang G, Chepurny OG, Holz GG (2001). cAMP-regulated guanine nucleotide exchange factor II (Epac2) mediates Ca2+-induced Ca2+ release in INS-1 pancreatic β-cells. J Physiol (Lond) 536: 375–385.

Kang G, Leech CA, Chepurny OG, Coetzee WA, Holz GG (2008). Role of the cAMP sensor Epac as a determinant of KATP channel ATP sensitivity in human pancreatic β-cells and rat INS-1 cells. J Physiol (Lond) 586: 1307–1319.

Kanoski SE, Fortin SM, Arnold M, Grill HJ, Hayes MR (2011). Peripheral and central GLP-1 receptor populations mediate the anorectic effects of peripherally administered GLP-1 receptor agonists, liraglutide and exendin-4. Endocrinology 152: 3103–3112.

Kanse SM, Kreymann B, Ghatei MA, Bloom SR (1988). ScienceDirect.com – FEBS Letters – Identification and characterization of glucagon-like peptide-1 7–36 amide-binding sites in the rat brain and lung. FEBS Lett 241: 209–212.

Kato T, Nagatsu T, Fukasawa K, Harada M, Nagatsu I, Sakakibara S (1978). Successive cleavage of N-terminal Arg1–Pro2 and Lys3-Pro4 from substance P but no release of Arg1-Pro2 from bradykinin, by X-Pro dipeptidyl-aminopeptidase. Biochim Biophys Acta 525: 417–422.

Kemp DM, Habener JF (2001). Insulinotropic hormone glucagon-like peptide 1 (GLP-1) activation of insulin gene promoter inhibited by p38 mitogen-activated protein kinase. Endocrinology 142: 1179–1187.

Kenny AJ, Booth AG, George SG, Ingram J, Kershaw D, Wood EJ, Young AR (1976). Dipeptidyl peptidase IV, a kidney brush-border serine peptidase. Biochem J 157: 169–182.

Kieffer TJ, McIntosh CH, Pederson RA (1995). Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 in vitro and in vivo by dipeptidyl peptidase IV. Endocrinology 136: 3585–3596.

Kim D, Wang L, Beconi M, Eiermann GJ, Fisher MH, He H *et al*. (2005). (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo [4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine: a potent, orally active dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. J Med Chem 48: 141–151.

Knudsen LB, Pridal L (1996). Glucagon-like peptide-1-(9-36) amide is a major metabolite of glucagon-like peptide-1-(7-36) amide after in vivo administration to dogs, and it acts as an antagonist on the pancreatic receptor. Eur J Pharmacol 318: 429–435.

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 J, Bessler M, Inabnet W, Taveras C, Holst JJ (2007). Exaggerated glucagon-like peptide-1 and blunted glucose-dependent insulinotropic peptide secretion are associated with Roux-en-Y gastric bypass but not adjustable gastric banding. Surg Obes Relat Dis 3: 597–601.

Körner M, Stöckli 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.

Kreymann B, Williams G, Ghatei MA, Bloom SR (1987). Glucagon-like peptide-1 7-36: a physiological incretin in man. Lancet 2: 1300–1304.

Lambeir AM, Durinx C, Proost P, Van Damme J, Scharpé S, De Meester I (2001). Kinetic study of the processing by dipeptidyl-peptidase IV/CD26 of neuropeptides involved in pancreatic insulin secretion. FEBS Lett 507: 327–330.

Lankat-Buttgereit B, Göke R, Stöckmann F, Jiang J, Fehmann HC, Göke B (1994). Detection of the human glucagon-like peptide 1(7-36) amide receptor on insulinoma-derived cell membranes. Digestion 55: 29–33.

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.

Lausier J, Diaz WC, Roskens V, LaRock K, Herzer K, Fong CG *et al.* (2010). Vagal control of pancreatic β-cell proliferation. Am J Physiol Endocrinol Metab 299: E786–E793.

Lawrence MC, Bhatt HS, Easom RA (2002). NFAT regulates insulin gene promoter activity in response to synergistic pathways induced by glucose and glucagon-like peptide-1. Diabetes 51: 691–698.

Light PE (2002). Glucagon-like peptide-1 inhibits pancreatic ATP-sensitive potassium channels via a protein kinase A- and ADP-dependent mechanism. Mol Endocrinol 16: 2135–2144.

Linnebjerg H, Park S, Kothare PA, Trautmann ME, Mace K, Fineman M *et al.* (2008). Effect of exenatide on gastric emptying and relationship to postprandial glycemia in type 2 diabetes. Regul Pept 151: 123–129.

Llewellyn-Smith IJ, Verberne AJM (2013). Central Regulation of Autonomic Functions. Oxford University Press: Oxford.

Lockie SH, Heppner KM, Chaudhary N, Chabenne JR, Morgan DA, Veyrat-Durebex C *et al.* (2012). Direct control of brown adipose tissue thermogenesis by central nervous system glucagon-like peptide-1 receptor signaling. Diabetes 61: 2753–2762.

Luque MA, González N, Márquez L, Acitores A, Redondo A, Morales M *et al.* (2002). Glucagon-like peptide-1 (GLP-1) and glucose metabolism in human myocytes. J Endocrinol 173: 465–473.

McIntyre N, Holdsworth CD, Turner DS (1964). New interpretation of oral glucose tolerance. Lancet 2: 20–21.

Malm-Erjefält M, Bjørnsdottir I, Vanggaard J, Helleberg H, Larsen U, Oosterhuis B *et al.* (2010). Metabolism and excretion of the once-daily human glucagon-like peptide-1 analog liraglutide in healthy male subjects and its in vitro degradation by dipeptidyl peptidase IV and neutral endopeptidase. Drug Metab Dispos 38: 1944–1953.

Matschinsky F, Liang Y, Kesavan P, Wang L, Froguel P, Velho G *et al.* (1993). Glucokinase as pancreatic beta cell glucose sensor and diabetes gene. J Clin Invest 92: 2092.

Matschinsky FM, Glaser B, Magnuson MA (1998). Pancreatic beta-cell glucokinase: closing the gap between theoretical concepts and experimental realities. Diabetes 47: 307–315.

Meier JJ, Nauck MA, Kranz D, Holst JJ, Deacon CF, Gaeckler D *et al.* (2004). Secretion, degradation, and elimination of glucagon-like peptide 1 and gastric inhibitory polypeptide in patients with chronic renal insufficiency and healthy control subjects. Diabetes 53: 654–662.

Meier JJ, Kemmeries G, Holst JJ, Nauck MA (2005). Erythromycin antagonizes the deceleration of gastric emptying by glucagon-like peptide 1 and unmasks its insulinotropic effect in healthy subjects. Diabetes 54: 2212–2218.

Meier JJ, Gethmann A, Nauck MA, Götze O, Schmitz F, Deacon CF *et al.* (2006). The glucagon-like peptide-1 metabolite GLP-1-(9-36) amide reduces postprandial glycemia independently of gastric emptying and insulin secretion in humans. Am J Physiol Endocrinol Metab 290: E1118–E1123.

Meneilly GS, McIntosh CHS, Pederson RA, Habener JF, Ehlers MRW, Egan JM, Elahi D (2003). Effect of glucagon-like peptide 1 (7-36 amide) on insulin-mediated glucose uptake in patients with type 1 diabetes. Diabetes Care 26: 837–842.

Mentlein R, Dahms P, Grandt D, Krüger R (1993a). Proteolytic processing of neuropeptide Y and peptide YY by dipeptidyl peptidase IV. Regul Pept 49: 133–144.

Mentlein R, Gallwitz B, Schmidt WE (1993b). 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.

Moens K, Heimberg H, Flamez D, Huypens P, Quartier E, Ling Z *et al.* (1996). Expression and functional activity of glucagon, glucagon-like peptide I, and glucose-dependent insulinotropic peptide receptors in rat pancreatic islet cells. Diabetes 45: 257–261.

Mojsov S, Heinrich G, Wilson IB, Ravazzola M, Orci L, Habener JF (1986). Preproglucagon gene expression in pancreas and intestine diversifies at the level of post-translational processing. J Biol Chem 261: 11880–11889.

Mojsov S, Weir GC, Habener JF (1987). Insulinotropin: glucagon-like peptide I (7-37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. J Clin Invest 79: 616–619.

Montrose-Rafizadeh C, Yang H, Rodgers BD, Beday A, Pritchette LA, Eng J (1997a). High potency antagonists of the pancreatic glucagon-like peptide-1 receptor. J Biol Chem 272: 21201–21206.

Montrose-Rafizadeh C, Yang H, Wang Y, Roth J, Montrose MH, Adams LG (1997b). Novel signal transduction and peptide specificity of glucagon-like peptide receptor in 3T3-L1 adipocytes. J Cell Physiol 172: 275–283.

Nachnani JS, Bulchandani DG, Nookala A, Herndon B, Molteni A, Pandya P *et al.* (2010). Biochemical and histological effects of exendin-4 (exenatide) on the rat pancreas. Diabetologia 53: 153–159.

Nagell CF, Wettergren A, Ørskov 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: E808–E813.

Nakagawa A, Satake H, Nakabayashi H, Nishizawa M, Furuya K, Nakano S *et al.* (2004). Receptor gene expression of glucagon-like peptide-1, but not glucose-dependent insulinotropic polypeptide, in rat nodose ganglion cells. Auton Neurosci 110: 36–43.

Näslund E, Gutniak M, Skogar S, Rössner S, Hellström PM (1998). Glucagon-like peptide 1 increases the period of postprandial satiety and slows gastric emptying in obese men. Am J Clin Nutr 68: 525–530.

Nauck MA, Bartels E, Orskov C, Ebert R, Creutzfeldt W (1993a). Additive insulinotropic effects of exogenous synthetic human gastric inhibitory polypeptide and glucagon-like peptide-1-(7-36)

amide infused at near-physiological insulinotropic hormone and glucose concentrations. J Clin Endocrinol Metab 76: 912–917.

Nauck MA, Heimesaat MM, Orskov C, Holst JJ, Ebert R, Creutzfeldt W (1993b). Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. J Clin Invest 91: 301–307.

Nauck MA, Kemmeries G, Holst JJ, Meier JJ (2011). Rapid tachyphylaxis of the glucagon-like peptide 1-induced deceleration of gastric emptying in humans. Diabetes 60: 1561–1565.

Nikolaidis LA, Elahi D, Shen Y-T, Shannon RP (2005). Active metabolite of GLP-1 mediates myocardial glucose uptake and improves left ventricular performance in conscious dogs with dilated cardiomyopathy. Am J Physiol Heart Circ Physiol 289: H2401–H2408.

Nishizawa M, Nakabayashi H, Uchida K, Nakagaw A, Niijima A (1996). The hepatic vagal nerve is receptive to incretin hormone glucagon-like peptide-l, but not to glucose-dependent insulinotropic polypeptide, in the portal vein. J Auton Nerv Syst 61: 149–154.

Nogueiras R, Perez-Tilve D, Veyrat-Durebex C, Morgan DA, Varela L, Haynes WG *et al.* (2009). Direct control of peripheral lipid deposition by CNS GLP-1 receptor signaling is mediated by the sympathetic nervous system and blunted in diet-induced obesity. J Neurosci 29: 5916–5925.

Oravecz T, Pall M, Roderiquez G, Gorrell MD, Ditto M, Nguyen NY *et al.* (1997). Regulation of the receptor specificity and function of the chemokine RANTES (regulated on activation, normal T cell expressed and secreted) by dipeptidyl peptidase IV (CD26)-mediated cleavage. J Exp Med 186: 1865–1872.

Orskov C, Poulsen SS (1991). Glucagonlike peptide-I-(7-36)-amide receptors only in islets of Langerhans. Autoradiographic survey of extracerebral tissues in rats. Diabetes 40: 1292–1296.

Orskov C, Holst JJ, Poulsen SS, Kirkegaard P (1987). Pancreatic and intestinal processing of proglucagon in man. Diabetologia 30: 874–881.

Orskov C, Rabenhøj L, Wettergren A, Kofod H, Holst JJ (1994). Tissue and plasma concentrations of amidated and glycine-extended glucagon-like peptide I in humans. Diabetes 43: 535–539.

Orskov C, Poulsen SS, Møller M, Holst JJ (1996a). 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.

Orskov L, Holst JJ, Møller J, Orskov C, Møller N, Alberti KG, Schmitz O (1996b). GLP-1 does not acutely affect insulin sensitivity in healthy man. Diabetologia 39: 1227–1232.

Ozaki N, Shibasaki T, Kashima Y, Miki T, Takahashi K, Ueno H *et al.* (2000). cAMP-GEFII is a direct target of cAMP in regulated exocytosis. Nat Cell Biol 2: 805–811.

Panjwani N, Mulvihill EE, Longuet C, Yusta B, Campbell JE, Brown TJ *et al.* (2013). GLP-1 receptor activation indirectly reduces hepatic lipid accumulation but does not attenuate development of atherosclerosis in diabetic male ApoE-/- mice. Endocrinology 154: 127–139.

Park JH, Kim SJ, Park SH, Son DG, Bae JH, Kim HK *et al.* (2012). Glucagon-like peptide-1 enhances glucokinase activity in pancreatic β-cells through the association of Epac2 with Rim2 and Rab3A. Endocrinology 153: 574–582.

Patzelt C, Tager HS, Carroll RJ, Steiner DF (1979). Identification and processing of proglucagon in pancreatic islets. Nature 282: 260–266.

Perfetti R, Zhou J, Doyle ME, Egan JM (2000). Glucagon-like peptide-1 induces cell proliferation and pancreatic-duodenum homeobox-1 expression and increases endocrine cell mass in the pancreas of old, glucose-intolerant rats. Endocrinology 141: 4600–4605.

Plamboeck A, Holst JJ, Carr RD, Deacon CF (2005). Neutral endopeptidase 24.11 and dipeptidyl peptidase IV are both mediators of the degradation of glucagon-like peptide 1 in the anaesthetised pig. Diabetologia 48: 1882–1890.

Pocai A (2012). Unraveling oxyntomodulin, GLP1's enigmatic brother. J Endocrinol 215: 335–346.

Pratley R, Nauck M, Bailey T, Montanya E, Cuddihy R, Filetti S *et al.* (2011). One year of liraglutide treatment offers sustained and more effective glycaemic control and weight reduction compared with sitagliptin, both in combination with metformin, in patients with type 2 diabetes: a randomised, parallel-group, open-label trial. Int J Clin Pract 65: 397–407.

Pratley RE, Nauck M, Bailey T, Montanya E, Cuddihy R, Filetti S *et al.* (2010). Liraglutide versus sitagliptin for patients with type 2 diabetes who did not have adequate glycaemic control with metformin: a 26-week, randomised, parallel-group, open-label trial. Lancet 375: 1447–1456.

Preitner F, Ibberson M, Franklin I, Binnert C, Pende M, Gjinovci A *et al.* (2004). Gluco-incretins control insulin secretion at multiple levels as revealed in mice lacking GLP-1 and GIP receptors. J Clin Invest 113: 635–645.

Prigeon RL, Quddusi S, Paty B, D'Alessio DA (2003). Suppression of glucose production by GLP-1 independent of islet hormones: a novel extrapancreatic effect. Am J Physiol Endocrinol Metab 285: E701–E707.

Proost P, Struyf S, Schols D, Durinx C, Wuyts A, Lenaerts JP *et al.* (1998). Processing by CD26/dipeptidyl-peptidase IV reduces the chemotactic and anti-HIV-1 activity of stromal-cell-derived factor-1alpha. FEBS Lett 432: 73–76.

Proost P, Struyf S, Schols D, Opdenakker G, Sozzani S, Allavena P *et al.* (1999). Truncation of macrophage-derived chemokine by CD26/ dipeptidyl-peptidase IV beyond its predicted cleavage site affects chemotactic activity and CC chemokine receptor 4 interaction. J Biol Chem 274: 3988–3993.

Proost P, Menten P, Struyf S, Schutyser E, De Meester I, Van Damme J (2000). Cleavage by CD26/dipeptidyl peptidase IV converts the chemokine LD78beta into a most efficient monocyte attractant and CCR1 agonist. Blood 96: 1674–1680.

Quoyer J, Longuet C, Broca C, Linck N, Costes S, Varin E *et al.* (2010). GLP-1 mediates antiapoptotic effect by phosphorylating Bad through a beta-arrestin 1-mediated ERK1/2 activation in pancreatic beta-cells. J Biol Chem 285: 1989–2002.

Reid T (2012). Choosing GLP-1 receptor agonists or DPP-4 inhibitors: weighing the clinical trial evidence. Clin Diabetes 30: 3–12.

Renner E, Puskás N, Dobolyi A, Palkovits M (2012). Glucagon-like peptide-1 of brainstem origin activates dorsomedial hypothalamic neurons in satiated rats. Peptides 35: 14–22.

Richter G, Göke R, Göke B, Arnold R (1990). Characterization of receptors for glucagon-like peptide-1(7-36)amide on rat lung membranes. FEBS Lett 267: 78–80.

Richter G, Feddersen O, Wagner U, Barth P, Göke R, Göke B (1993). GLP-1 stimulates secretion of macromolecules from airways and relaxes pulmonary artery. Am J Physiol 265: L374–L381.

Rolin B, Larsen MO, Gotfredsen CF, Deacon CF, Carr RD, Wilken M, Knudsen LB (2002). The long-acting GLP-1 derivative NN2211 ameliorates glycemia and increases beta-cell mass in diabetic mice. Am J Physiol Endocrinol Metab 283: E745–E752.

Rosenstock J, Reusch J, Bush M, Yang F, Stewart M, Albiglutide Study Group (2009). Potential of albiglutide, a long-acting GLP-1 receptor agonist, in type 2 diabetes: a randomized controlled trial exploring weekly, biweekly, and monthly dosing. Diabetes Care 32: 1880–1886.

Rosenstock J, Balas B, Charbonnel B, Bolli GB, Boldrin M, Ratner R *et al.* (2013). The fate of taspoglutide, a weekly GLP-1 receptor agonist, versus twice-daily exenatide for type 2 diabetes: the T-emerge 2 trial. Diabetes Care 36: 498–504.

Ruiz-Grande C, Alarcón C, Alcántara A, Castilla C, López Novoa JM, Villanueva-Peñacarrillo ML, Valverde I (1993). Renal catabolism of truncated glucagon-like peptide 1. Horm Metab Res 25: 612–616.

Sancho V, Nuche B, Arnés L, Cancelas J, González N, Díaz-Miguel M *et al.* (2007). The action of GLP-1 and exendins upon glucose transport in normal human adipocytes, and on kinase activity as compared to morbidly obese patients. Int J Mol Med 19: 961–966.

Sandoval DA, Bagnol D, Woods SC, D'Alessio DA, Seeley RJ (2008). Arcuate glucagon-like peptide 1 receptors regulate glucose homeostasis but not food intake. Diabetes 57: 2046–2054.

Scheen AJ (2012). DPP-4 inhibitors in the management of type 2 diabetes: a critical review of head-to-head trials. Diabetes Metab 38: 89–101.

Schepp W, Dehne K, Riedel T, Schmidtler J, Schaffer K, Classen M (1996). Oxyntomodulin: a cAMP-dependent stimulus of rat parietal cell function via the receptor for glucagon-like peptide-1 (7-36)NH2. Digestion 57: 398–405.

Schirra J, Wank U, Arnold R, Göke B, Katschinski M (2002). Effects of glucagon-like peptide-1(7-36)amide on motility and sensation of the proximal stomach in humans. Gut 50: 341–348.

Schirra J, Nicolaus M, Roggel R, Katschinski M, Storr M, Woerle HJ, Göke B (2006). Endogenous glucagon-like peptide 1 controls endocrine pancreatic secretion and antro-pyloro-duodenal motility in humans. Gut 55: 243–251.

Schlatter P, Beglinger C, Drewe J, Gutmann H (2007). Glucagon-like peptide 1 receptor expression in primary porcine proximal tubular cells. Regul Pept 141: 120–128.

Schuit FC, Huypens P, Heimberg H, Pipeleers DG (2001). Glucose sensing in pancreatic β-cells a model for the study of other glucose-regulated cells in gut, pancreas, and hypothalamus. Diabetes 50: 1–11.

Scrocchi LA, Brown TJ, MaClusky N, Brubaker PL, Auerbach AB, Joyner AL, Drucker DJ (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.

Seghieri M, Rebelos E, Gastaldelli A, Astiarraga BD, Casolaro A, Barsotti E *et al.* (2013). Direct effect of GLP-1 infusion on endogenous glucose production in humans. Diabetologia 56: 156–161.

Shibasaki T, Takahashi H, Miki T, Sunaga Y, Matsumura K, Yamanaka M *et al.* (2007). Essential role of Epac2/Rap1 signaling in regulation of insulin granule dynamics by cAMP. Proc Natl Acad Sci U S A 104: 19333–19338.

Shughrue PJ, Lane MV, Merchenthaler I (1996). Glucagon-like peptide-1 receptor (GLP1-R) mRNA in the rat hypothalamus. Endocrinology 137: 5159–5162.

Singh S, Chang H-Y, Richards TM, Weiner JP, Clark JM, Segal JB (2013). Glucagonlike peptide 1-based therapies and risk of hospitalization for acute pancreatitis in type 2 diabetes mellitus: a population-based matched case-control study. JAMA Intern Med 173: 534–539.

Skoglund G, Hussain MA, Holz GG (2000). Glucagon-like peptide 1 stimulates insulin gene promoter activity by protein kinase A-independent activation of the rat insulin I gene cAMP response element. Diabetes 49: 1156–1164.

Song W-J, Seshadri M, Ashraf U, Mdluli T, Mondal P, Keil M *et al.* (2011). Snapin mediates incretin action and augments glucose-dependent insulin secretion. Cell Metab 13: 308–319.

Sonoda N, Imamura T, Yoshizaki T, Babendure JL, Lu J-C, Olefsky JM (2008). Beta-Arrestin-1 mediates glucagon-like peptide-1 signaling to insulin secretion in cultured pancreatic beta cells. Proc Natl Acad Sci U S A 105: 6614–6619.

Stoffers DA, Kieffer TJ, Hussain MA, Drucker DJ, Bonner-Weir S, Habener JF, Egan JM (2000). Insulinotropic glucagon-like peptide 1 agonists stimulate expression of homeodomain protein IDX-1 and increase islet size in mouse pancreas. Diabetes 49: 741–748.

Sturis J, Gotfredsen CF, Rømer J, Rolin B, Ribel U, Brand CL *et al.* (2003). GLP-1 derivative liraglutide in rats with beta-cell deficiencies: influence of metabolic state on beta-cell mass dynamics. Br J Pharmacol 140: 123–132.

Tang-Christensen M, Larsen PJ, Göke R, Fink-Jensen A, Jessop DS, Møller M, Sheikh SP (1996). Central administration of GLP-1-(7-36) amide inhibits food and water intake in rats. Am J Physiol 271: R848–R856.

Tomas E, Habener JF (2010). Insulin-like actions of glucagon-like peptide-1: a dual receptor hypothesis. Trends Endocrinol Metab 21: 59–67.

Tornehave D, Kristensen P, Rømer J, Knudsen LB, Heller RS (2008). Expression of the GLP-1 receptor in mouse, rat, and human pancreas. J Histochem Cytochem 56: 841–851.

Tourrel C, Bailbé D, Meile MJ, Kergoat M, Portha B (2001). Glucagon-like peptide-1 and exendin-4 stimulate beta-cell neogenesis in streptozotocin-treated newborn rats resulting in persistently improved glucose homeostasis at adult age. Diabetes 50: 1562–1570.

Turton MD, O'Shea D, Gunn I, Beak SA, Edwards CM, Meeran K *et al.* (1996). A role for glucagon-like peptide-1 in the central regulation of feeding. Nature 379: 69–72.

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.

Vella A, Shah P, Basu R, Basu A, Holst JJ, Rizza RA (2000). Effect of glucagon-like peptide 1(7-36) amide on glucose effectiveness and insulin action in people with type 2 diabetes. Diabetes 49: 611–617.

Vella A, Shah P, Reed A, Adkins A, Basu R, Rizza R (2002). Lack of effect of exendin-4 and glucagon-like peptide-1-(7, 36)-amide on insulin action in non-diabetic humans. Diabetologia 45: 1410–1415.

Villanueva-Peñacarrillo ML, Delgado E, Trapote MA, Alcántara A, Clemente F, Luque MA *et al.* (1995). Glucagon-like peptide-1 binding to rat hepatic membranes. J Endocrinol 146: 183–189.

Wan S, Browning KN, Travagli RA (2007a). Glucagon-like peptide-1 modulates synaptic transmission to identified pancreas-projecting vagal motoneurons. Peptides 28: 2184–2191.

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

Wang A, Dorso C, Kopcho L, Locke G, Langish R, Harstad E *et al.* (2012). Potency, selectivity and prolonged binding of saxagliptin to DPP4: maintenance of DPP4 inhibition by saxagliptin in vitro and ex vivo when compared to a rapidly-dissociating DPP4 inhibitor. BMC Pharmacol 12: 2.

Wang Q, Brubaker PL (2002). Glucagon-like peptide-1 treatment delays the onset of diabetes in 8 week-old db/db mice. Diabetologia 45: 1263–1273.

Wang X, Zhou J, Doyle ME, Egan JM (2001). Glucagon-like peptide-1 causes pancreatic duodenal homeobox-1 protein translocation from the cytoplasm to the nucleus of pancreatic β-cells by a cyclic adenosine monophosphate/protein kinase A-dependent mechanism. Endocrinology 142: 1820–1827.

Wang Y, Egan JM, Raygada M, Nadiv O, Roth J, Montrose-Rafizadeh C (1995). Glucagon-like peptide-1 affects gene transcription and messenger ribonucleic acid stability of components of the insulin secretory system in RIN 1046-38 cells. Endocrinology 136: 4910–4917.

Wang Y, Kole HK, Montrose-Rafizadeh C, Perfetti R, Bernier M, Egan JM (1997). Regulation of glucose transporters and hexose uptake in 3T3-L1 adipocytes: glucagon-like peptide-1 and insulin interactions. J Mol Endocrinol 19: 241–248.

Washington MC, Raboin SJ, Thompson W, Larsen CJ, Sayegh AI (2010). Exenatide reduces food intake and activates the enteric nervous system of the gastrointestinal tract and the dorsal vagal complex of the hindbrain in the rat by a GLP-1 receptor. Brain Res 1344: 124–133.

Wettergren A, Petersen H, Orskov C, Christiansen J, Sheikh SP, Holst JJ (1994). Glucagon-like peptide-1 7-36 amide and peptide YY from the L-cell of the ileal mucosa are potent inhibitors of vagally induced gastric acid secretion in man. Scand J Gastroenterol 29: 501–505.

Wettergren A, Wøjdemann 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.

Wettergren A, Wøjdemann M, Holst JJ (1998). Glucagon-like peptide-1 inhibits gastropancreatic function by inhibiting central parasympathetic outflow. Am J Physiol 275: G984–G992.

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

Xie T, Chen M, Zhang Q-H, Ma Z, Weinstein LS (2007). Beta cell-specific deficiency of the stimulatory G protein alpha-subunit Gsalpha leads to reduced beta cell mass and insulin-deficient diabetes. Proc Natl Acad Sci U S A 104: 19601–19606.

Xu G, Stoffers DA, Habener JF, Bonner-Weir S (1999). Exendin-4 stimulates both beta-cell replication and neogenesis, resulting in increased beta-cell mass and improved glucose tolerance in diabetic rats. Diabetes 48: 2270–2276.

Xu G, Kaneto H, Lopez-Avalos MD, Weir GC, Bonner-Weir S (2006). GLP-1/exendin-4 facilitates beta-cell neogenesis in rat and human pancreatic ducts. Diabetes Res Clin Pract 73: 107–110.

Yamada C, Yamada Y, Tsukiyama K, Yamada K, Udagawa N, Takahashi N *et al.* (2008). The murine glucagon-like peptide-1 receptor is essential for control of bone resorption. Endocrinology 149: 574–579.

Yamamoto H, Lee CE, Marcus JN, Williams TD, Overton JM, Lopez ME *et al.* (2002). Glucagon-like peptide-1 receptor stimulation increases blood pressure and heart rate and activates autonomic regulatory neurons. J Clin Invest 110: 43–52.

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.

Zander M, Madsbad S, Madsen JL, Holst JJ (2002). Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and β-cell function in type 2 diabetes: a parallel-group study. Lancet 359: 824–830.