REVIEW

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

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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, intracerebroventricular; 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 haemoglobin)

	Sulfonyl-ureas	Metformin	TZDs	DPPIV inhibitors	GLP-1 mimetics	
					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 diabetes (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 neprylisin in humans (Malm-Erfjält *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 immunofluorescence (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 immunofluorescent 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(1-36NH₂), 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_{as}) 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 phosphorylation 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 phosphorylating 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 V_{max}) 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 pancreaticoduodenum 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 ana-

logues 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)NH₂) 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 amino acid polylysine extension at the C-terminus, have circulating half lives of around 4 h but have been formulated

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).

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 position 8 valine substituted GLP-1 sequence fused via a linker to human IgG4-Fc domain and has a half life of 4 days. 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 activation and do not suffer from the potential off target effects of DPPIV inhibitors. Clinically, all GLP-1 mimetics are better at lowering fasting and postprandial plasma glucose, glycosylated haemoglobin 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 receptor 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 neoplasia 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.

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Conflict of interest

None declared.

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