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Themed Section: Secretin Family (Class B) G Protein-Coupled Receptors – from Molecular to Clinical Perspectives

REVIEW

The structure and function of the glucagon-like peptide-1 receptor and its ligands

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Glucagon-like peptide-1(7-36)amide (GLP-1) is a 30-residue peptide hormone released from intestinal L cells following nutrient consumption. It potentiates the glucose-induced secretion of insulin from pancreatic beta cells, increases insulin expression, inhibits beta-cell apoptosis, promotes beta-cell neogenesis, reduces glucagon secretion, delays gastric emptying, promotes satiety and increases peripheral glucose disposal. These multiple effects have generated a great deal of interest in the discovery of long-lasting agonists of the GLP-1 receptor (GLP-1R) in order to treat type 2 diabetes. This review article summarizes the literature regarding the discovery of GLP-1 and its physiological functions. The structure, function and sequence–activity relationships of the hormone and its natural analogue exendin-4 (Ex4) are reviewed in detail. The current knowledge of the structure of GLP-1R, a Family B GPCR, is summarized and discussed, before its known interactions with the principle peptide ligands are described and summarized. Finally, progress in discovering non-peptide ligands of GLP-1R is reviewed. GLP-1 is clearly an important hormone linking nutrient consumption with blood sugar control, and therefore knowledge of its structure, function and mechanism of action is of great importance.

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Abbreviations
BPh, benzoylphenylalanine; DPPIV, dipeptidyl peptidase IV; DSS, disuccinimidyl suberate; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; GLP-1R, GLP-1 receptor; hGLP-1R, human GLP-1R; NTD, N-terminal domain; rGLP-1R, rat GLP-1R

Introduction

The incretin effect describes the increased secretory response of beta cells, above that of glycaemia itself, which results from the actions of gut-derived factors (McIntyre *et al.*, 1964). Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are the hormones responsible for the incretin effect, and together they account for up to 60% of postprandial insulin release (Nauck *et al.*, 1986). However, despite their equivalent roles in potentiating glucose-induced insulin release, therapeutic strategies for the treatment of type 2 diabetes have focused primarily on GLP-1 since, unlike GIP, it continues to elicit a response in type 2 diabetic patients (Nauck *et al.*, 1993).

GLP-1 is expressed in intestinal L cells and is derived from the cell-specific post-translational processing of the preproglucagon gene (Mojsov *et al.*, 1986). Initially, the peptide GLP-1(1-37) was identified from this processing, but it was the two N-terminally truncated products, GLP-1(7-37) and GLP-1(7-36)amide, that were found to recognize the pancreatic receptor and which were determined to be the active species *in vivo* (Drucker *et al.*, 1987; Holst *et al.*, 1987; Mojsov *et al.*, 1987; Ørskov and Nielsen, 1988; Weir *et al.*, 1989). These two shorter peptides are equipotent (Ørskov *et al.*, 1993) and are the major physiological incretin in humans (Edwards *et al.*, 1999); hence, these N-terminally truncated peptides are the forms implied by the generic term 'GLP-1' throughout this review.

The mechanisms of action of GLP-1 (reviewed extensively by Doyle and Egan, 2007) go beyond just the augmentation of glucose-induced insulin secretion and include increasing insulin expression (Drucker *et al.*, 1987), inhibiting beta-cell

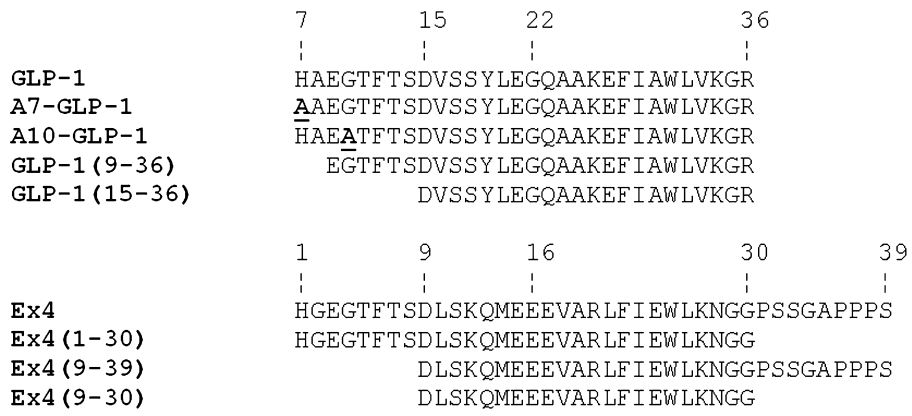

Figure 1

A sequence alignment of GLP-1, Ex4 and several analogues mentioned at various stages in the text. All peptides are C-terminally amidated. Note that the first residue of GLP-1 is His^{7}, while that of Ex4 is His^{1}. Residues changed from the original sequence are underlined.

apoptosis and promoting beta-cell neogenesis (Perfetti and Hui, 2004; Cornu and Thorens, 2009); reducing glucagon secretion (Kreymann *et al.*, 1987), delaying gastric emptying (Wettergren *et al.*, 1993; Nauck *et al.*, 1997), promoting satiety (Turton *et al.*, 1996; Flint *et al.*, 1998) and increasing peripheral glucose disposal (D'Alessio *et al.*, 1994; 1995; Egan *et al.*, 2002). It is clear from these varied actions that GLP-1 plays a central role in controlling postprandial blood sugar levels, and hence, the enhancement of GLP-1 action has become an important area for the development of new therapies to treat type 2 diabetes (e.g. Ahrén, 2011). GLP-1 raises insulin and lowers glucagon in type 2 diabetic patients (Nathan *et al.*, 1992; Nauck *et al.*, 1993), and repeated s.c. injections were found to normalize plasma glucose levels (Nauck *et al.*, 1996). However, the half-life of GLP-1 *in vivo* is short, and it is rapidly degraded to GLP-1(9-36)amide by DPPIV (Deacon *et al.*, 1995; Knudsen and Pridal, 1996; Hansen *et al.*, 1999), and hence, there are continuing attempts to find long-lasting peptide agonists with glucoregulatory properties (e.g. Gong *et al.*, 2011). Indeed, current therapeutic approaches have utilized DPPIV-resistant mimetics of GLP-1, most notably exenatide (Iltz *et al.*, 2006) and liraglutide (Neumiller and Campbell, 2009), both of which are in current therapeutic use. Exenatide is the synthetic form of the naturally occurring peptide exendin-4 (Ex4), originally isolated from the saliva of the Gila monster *Heloderma suspectum* (Eng *et al.*, 1992). It is 53% identical to GLP-1 but is resistant to DPPIV cleavage, largely due to the substitution of Ala^{2} by Gly (Figure 1). Liraglutide, on the other hand, is a synthetic analogue of GLP-1 itself, with minor sequence alterations and a covalently linked C-16 acyl chain.

The receptor for GLP-1 (GLP-1R) was first cloned from a cDNA library derived from rat pancreatic islets (Thorens, 1992), and the following year, the human receptor was cloned (Dillon *et al.*, 1993; Graziano *et al.*, 1993; Thorens *et al.*, 1993). The cloning revealed a receptor sequence of 463 residues that resembled the receptors for secretin, parathyroid hormone and calcitonin. These receptors formed a new branch of the GPCR superfamily, named 'Family B' (or 'Class B', or 'secretin receptor-like'), which to date includes 15 members (Hoare, 2005). These receptors possess a unique extracellular N-terminal domain (NTD) of 100–150 residues, which is connected to the integral membrane core domain (or J domain) that is typical of all GPCRs.

Family B GPCRs bind their peptide ligands via a common mechanism known as the 'two-domain model' (Bergwitz *et al.*, 1996; Hoare, 2005) in which the NTD first binds to the C-terminal helical region of the ligand, thereby enabling a second interaction between the N-terminal region of the ligand and the core domain of the receptor. The latter interaction is essential for enabling agonist-induced receptor activation. An alternative model to explain Family B GPCR activation has been proposed, in which the ligand-binding event induces a conformational change that exposes an 'endogenous agonist', which then interacts with the receptor core domain to initiate activity (Dong *et al.*, 2006; Dong *et al.*, 2008).

The importance of the ligand's α-helical secondary structure in the two-domain model for mediating the initial interaction with the NTD has been reviewed (Parthier *et al.*, 2009), while a common helix-capping model to explain how the various ligands of Family B GPCRs might activate their receptors has been proposed (Neumann *et al.*, 2008). An example of how knowledge of the two-domain model can be used to design and synthesize novel ligands has recently been described in an elegant study by Devigny *et al.* (2011), who produced high potency probes with *in vivo* activity. An azide-modified peptide based upon the C-terminus of the ligand, corticotropin releasing factor, was used as a carrier protein and fused to an acetylene-tagged peptide library from which the activating mimetic of the ligand's N-terminus was derived.

This article will review the structure and function of GLP-1R with particular emphasis upon how it interacts with its peptide ligands. In addition, the sequence– and structure–activity properties of the peptide ligands will be considered, as will the few non-peptide ligands that have been discovered to date.

Peptide ligands

GLP-1

Using perfused rat pancreas, Suzuki *et al*. (1989) demonstrated that the free N-terminal His^{7} was important for GLP-1's insulinotropic activity. While GLP-1(7-37) elicited a clear response at 0.1–1 nM, its N-terminally truncated analogue GLP-1(8-37) did not. Moreover, since GLP-1(1-37) and GLP-1(6-37) also failed to initiate activity, it suggested that His^{7} was required as the N-terminal residue of the agonist peptide. In contrast, truncation of the two extreme C-terminal residues (Arg^{36} and Gly^{37}) resulted in a less dramatic effect, although potency was nevertheless reduced. These sequence–activity data were in agreement with the findings of Mojsov (1992), using insulin-secreting RIN1046-38 cells. Radioligand binding demonstrated that the removal of C-terminal residues resulted in a 5- to 10-fold reduction in affinity, whereas removal of the N-terminal His^{7} [GLP-1(8-37)], or inclusion of the full N-terminal sequence [GLP-1(1-37)], resulted in greater than 300-fold reduced affinity. Removal of the first 14 residues of GLP-1 yielded a peptide with 1500-fold lower affinity than GLP-1 and with antagonist properties, while removal of the C-terminal 11 residues resulted in undetectable binding and activity (Gallwitz *et al*., 1990).

A residue-by-residue substitution of the GLP-1 sequence was carried out by Novo Nordisk in 1994 (Adelhorst *et al*., 1994). Each residue was individually substituted by Ala, unless it was already Ala in which case it was substituted by its equivalent in glucagon (Ala^{25} was not studied in this series, presumably because it is also Ala in glucagon). Adenylyl cyclase activity was used to assess agonist potency and intrinsic activity using membranes from RIN-2A18 cells, while competition binding assays were used to assess ligand affinity using membranes derived from Chinese hamster lung cells expressing recombinant rat GLP-1R (rGLP-1R). The binding assays suggested that the N-terminal positions 7, 10, 12, 13 and 15 were important for GLP-1 affinity at rGLP-1R since the substitutions resulted in a 41- to 219-fold increase in IC_{50}. A similar Ala-scan study in the same year, using RINm5F cells, identified the same five residues in the N-terminal region as being important for affinity (132- to 425-fold reduced affinity; Gallwitz *et al*., 1994). The His^{7} substitution by Ala resulted in a 111- to 374-fold reduction in affinity in these two studies, in contrast with only a 10-fold reduction at recombinant human GLP-1R (hGLP-1R) caused by replacing His^{7} by Tyr (Xiao *et al*., 2001). In another study using rGLP-1R expressed in Chinese hamster lymphoblast cells, His^{7} to Ala resulted in a 397-fold reduction in affinity, compared with 174-fold reduction by the Tyr substitution (Hareter *et al*., 1997). Furthermore, the His^{7} to Ala substitution resulted in a peptide that displayed no detectable adenylyl cyclase activity using 10 μM peptide, whereas the Tyr substituted peptide had a potency of 34 nM (GLP-1 control EC_{50} was 2.6 nM; Adelhorst *et al*., 1994; Xiao *et al*., 2001).

The substitution of Ala^{8} with D-Ala resulted in less than a twofold effect on affinity and less than a 10-fold reduction in potency at hGLP1-R (Xiao *et al*., 2001), while substitution by Ser resulted in a ninefold reduction in affinity at rGLP-1R, with no effect upon potency (Adelhorst *et al*., 1994). Therefore, Ala^{8} appears to be less important to the peptide's function compared with His^{7}. Truncation of both these residues to give GLP-1(9-36)amide (the natural DPPIV cleavage product) yielded a peptide with partial agonist activities. A study using transfected CHO cells expressing recombinant rGLP-1R demonstrated that GLP-1(9-36)amide displayed a 94-fold reduced affinity compared with GLP-1 and produced approximately 50% of the cAMP levels at 1 μM compared with that produced by 10 nM GLP-1 (Montrose-Rafizadeh *et al*., 1997). This partial agonist is present in the circulation at four to five times the level of GLP-1 (Egan *et al*., 2002), but initial studies did not demonstrate that it produced any biological activity (Vahl *et al*., 2003). However, it has since been suggested that GLP-1(9-36)amide is indeed insulinotropic, but that its actions are masked by the more potent effects of GLP-1 itself (Elahi *et al*., 2008).

The N-terminal region also contains other important residues and, indeed, the further truncation of GLP-1, to form GLP-1(15-36)amide, resulted in a 367-fold reduction in affinity (rGLP-1R in CHO cells) and no detectable activity at 1 μM peptide (Montrose-Rafizadeh *et al*., 1997). Although Glu-9 was not identified as being of particular importance by Adelhorst *et al*. (1994), Xiao *et al*. (2001) demonstrated that its substitution resulted in an 81-fold reduction in affinity and a 30-fold reduction in potency. Adelhorst *et al*. (1994) detected no adenylyl cyclase activity for GLP-1 peptides with either Gly^{10} or Asp^{15} substituted by Ala, while the Gly^{10} to Ala substitution by Xiao *et al*. (2001) resulted in more than 100-fold reduced affinity and greatly reduced potency. Watanabe *et al*. (1994) also studied position 10 and found that substitution by Ala resulted in a 221-fold reduction in affinity, while the D-Ala and β-Ala substitutions had less effect. Siegel *et al*. (1999) demonstrated that substitution of Ser^{14} and Asp^{15} resulted in significant decrease in affinity and activity at RINm5f cells.

An N-terminal fragment of GLP-1, corresponding to GLP-1(7-17) but with Val^{16} and Ser^{17} substituted with biphenylalanine derivatives, displayed potency that was only 206-fold lower than GLP-1 itself (Mapelli *et al*., 2009). Further modification by replacing Ala^{2} (by aminoisobutyric acid) and Phe^{6} (by α-methyl-phenylalanine derivative) led to the discovery of an 11-mer with potency that was only two to threefold lower than GLP-1. Hence the region of GLP-1 that is essential for activity must be located within positions 7–17. Therefore, although the two domain model suggests that the activating N-terminal region of the peptide agonists must be 'delivered' to the core domain via binding of the C-terminus to the NTD, this 11-mer agonist suggests that it is nevertheless possible to obtain high potency from the secondary interaction.

Adelhorst *et al*. (1994) and Gallwitz *et al*. (1994) also identified both Phe^{28} and Ile^{29} as being important for GLP-1's activity, probably due to their role in maintaining the helical structure of the peptide as assayed by far-UV CD spectroscopy (Adelhorst *et al*., 1994). However, many residues in GLP-1 appear not to be critical for affinity or activity, and indeed the discovery and analysis of a GLP-1-like peptide from *Xenopus* reveals that, despite 9 residue substitutions (Phe^{12}–Tyr, Ser^{14}–Asn, Ser^{17}–Thr, Ser^{18}–Glu, Gly^{22}–Glu, Gln^{23}–Lys, Ala^{30}–Glu, Val^{33}–Ile, Arg^{36}–Lys), its affinity and potency at hGLP-1R were nevertheless slightly better than GLP-1 itself (Irwin *et al*., 1997). The non-involvement of these residues in receptor affinity and efficacy is reflected in the results of the Ala scan

of Adelhorst *et al*. (1994) and Gallwitz *et al*. (1994) with the possible exception of Phe-^{12} (substitution to Ala resulted in 133-fold reduced affinity and 13-fold reduced potency), although the larger Tyr side chain in the *Xenopus* sequence is likely to be a more acceptable substitution compared with Ala.

Valuable insights into GLP-1 structure and function, as well as potential anti-obesity compounds, have come from studying the features that govern the selectivity between GLP-1 and glucagon at their related receptors. For example, the replacement of the C-terminal sequence 'VKGR' of GLP-1 with the corresponding 'MNT' of glucagon resulted in a 475-fold decrease in affinity. However, the substitution of residues in the N-terminal half of GLP-1 for those of glucagon had much smaller effects (Hjorth *et al*., 1994; Hjorth and Schwartz, 1996). Runge *et al*. (2003a,b) demonstrated, using chimaeric receptors, that the selectivity of GLP-1R for GLP-1 over glucagon is due to the NTD recognising the C-terminus of the peptide, while the toleration of GLP-1R for the glucagon N-terminal sequence results from the core domain. Understanding the specificity requirements for GLP-1 and glucagon at GLP-1R and the glucagon receptor has enabled the design of a co-agonist peptide that eliminates obesity in a rodent model (Day *et al*., 2009; Day *et al*., 2010). Full potency was obtained at GLP-1R using a peptide corresponding to glucagon but with changes at just three side chains (Ser16-Glu, Arg18-Ala, Gln20-Lys) and amidation of the C-terminus.

Exendin-4

The study of GLP-1R has been greatly enhanced by the discovery of the peptide agonist exendin-4 (Ex4) from the saliva of the Gila monster *H suspectum* (Eng *et al*., 1992). Although useful for elucidating aspects of GLP-1R's structure-function, Ex4 is not simply a research tool since, in its synthetic form, it constitutes the drug exenatide (Byetta; Iltz *et al*., 2006). It was actually the related peptide exendin-3, discovered in *H horridum*, which was discovered first and found to have efficacy in dispersed pancreatic acini (Eng *et al*., 1990). However, despite only differing by 2 of the 39 residues (Gly^2, Glu^3 in Ex4, compared with Ser^2, Asp^3 in exendin-3), Ex4 was found to have distinct pharmacological properties and does not act at VIP or secretin receptors. The N-terminal truncation of the first eight residues of exendin-3 yielded an antagonist that was initially used as a tool to elucidate the pharmacology of exendin-3 (Raufman *et al*., 1991). However, since exendin-3 and Ex4 are identical from residues 4–39, this antagonist, Ex4(9-39), has become a valuable tool for studying GLP-1R. Ex4 was found to share a similar pharmacological profile to GLP-1 (Raufman *et al*., 1992) being a potent GLP-1R agonist, while Ex4(9-39) is an antagonist (Göke *et al*., 1993; Thorens *et al*., 1993; Schepp *et al*., 1994; Kolligs *et al*., 1995). However, while usually described as an antagonist, Ex4(9-39) has also been described as an inverse agonist at the murine GLP-1R (Serre *et al*., 1998).

Despite their related sequences and similar pharmacological profile, sequence–activity studies using Ex4 have highlighted some clear differences with GLP-1. While the removal of the first two N-terminal residues of GLP-1 yielded a partial agonist with a 100-fold reduction in affinity, Ex4(3-39) has been reported to be a high-affinity antagonist (Montrose-Rafizadeh *et al*., 1997). Furthermore, while the further truncation of GLP-1 to remove the first eight residues resulted in a peptide with 370-fold reduced affinity [GLP-1(15-36)amide], Ex4(9-39) retains high affinity for GLP-1R. Hence, it is clear that, despite sharing eight of the nine residues at the N-terminus, these regions of the two peptides play a subtly different role in receptor interactions. For example, while the Gly2-Ala substitution in Ex4 is tolerated (Al-Sabah and Donnelly, 2003a; 2004; Patterson *et al*., 2011a), the converse Ala2-Gly change in GLP-1 reduces affinity by three- to fivefold (Deacon *et al*., 1998; Burcelin *et al*., 1999; Patterson *et al*., 2011a). Therefore, the interaction between the NTD and the C-terminal helix of the ligand can result in a different interaction between the N-terminus and the core domain (Al-Sabah and Donnelly, 2004). Indeed, substitution of Glu^{22}, Glu^{27} and Ala^{30} in GLP-1, with Gly, Leu and Glu from Ex4, enabled Gly^2 tolerance (Patterson *et al*., 2011a).

Interestingly, while Ex4(2-39) retains high affinity and full activity at 10 nM, the replacement of Asp^9 with Glu resulted in the complete loss of efficacy, despite no effect upon affinity (Montrose-Rafizadeh *et al*., 1997), suggesting that Asp^9 plays an important role in activity.

A large number of GLP-1/Ex4 chimaeric peptides have been utilized in order to understand the determinants that enable Ex4(9-39) to retain potent antagonist activity, while GLP-1(15-36) remains an agonist (Patterson *et al*., 2011b). Glu^{16}, Val^{19} and Arg^{20} were determined to be the essential determinants of Ex4(9-39)'s antagonism, in agreement with earlier chimaeric studies that demonstrated that the 'E^{16}EAVRL' sequence within the N-terminally truncated Ex4 was the determinant of its greater affinity over that of GLP-1(15-36)amide.

Updated data – a new view!

Recent data from the author's laboratory and in Patterson *et al*. (2011b) challenge some of the commonly held views about GLP-1 and Ex4 efficacy. The data reviewed in the sections above clearly point to the N-terminal region of GLP-1 as being essential for activity. However, using recombinant HEK-293 cell lines, it is possible to obtain robust activity for a number of peptide ligands previously believed to be devoid of efficacy (e.g. [His7-Ala]GLP-1; [Gly10-Ala]GLP-1; GLP-1(15-36)amide; Ex4(3-39); Figure 2; Patterson *et al*., 2011b). It is likely that the receptor reserve in the HEK-293 expression system enables the detection of agonists with low efficacy and therefore provides a useful environment in which to distinguish affinity-generating determinants from those responsible for creating efficacy.

Peptide structures

NMR analysis of GLP-1 in dodecylphosphocholine micelles revealed that, while the N-terminal residues 7–13 are unstructured, the rest of the peptide forms two helices from positions 13–20 and 24–35, separated by a linker region formed around Gly^{22} (Thornton and Gorenstein, 1994). Parker *et al*. (1998) utilized a number of cross-linked analogues of GLP-1 in order to trap an active conformation. The structures were largely compatible with the two-helix description of Thornton and Gorenstein. In contrast, Ex4 (which has Glu-16 in the equivalent position to Gly^{22} in GLP-1) forms a single helix spanning residues 11–27 (Neidigh *et al*., 2001). A recent NMR analysis

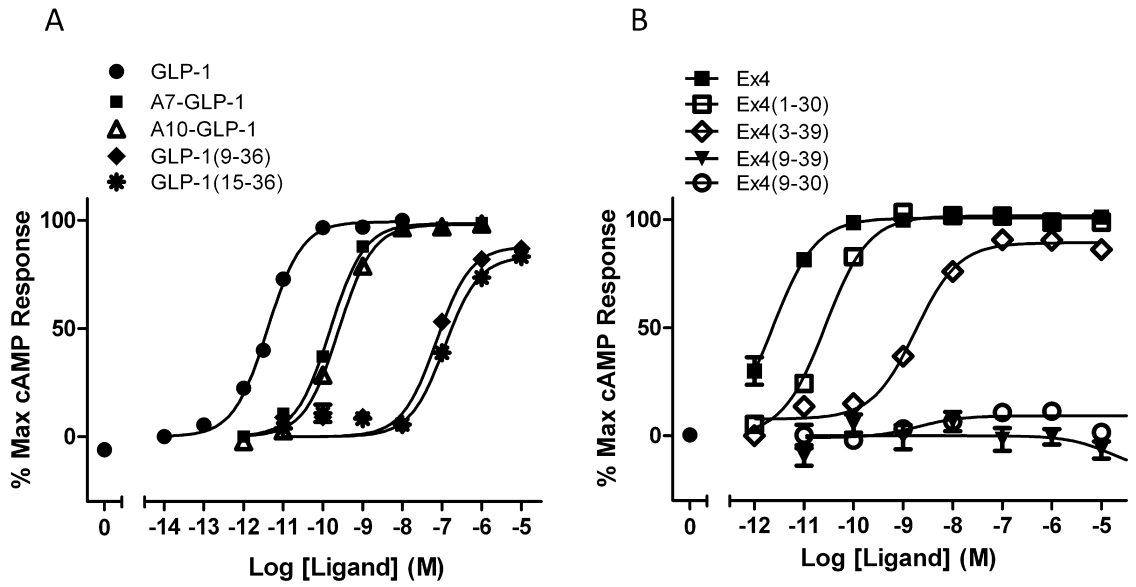

Figure 2

Concentration–response curves (LANCE cAMP assay) for various peptides at hGLP-1R, expressed in HEK-293 cells, using the same cell line as described previously by Mann *et al.* (2010a,b). pEC_{50} values, averaged over three independent experiments, were (A) GLP-1, 11.17 ± 0.12; A7-GLP-1,9.64 ± 0.09; A10-GLP-1, 9.45 ± 0.08; GLP-1(9-36), 6.97 ± 0.09; GLP-1(15-36), 6.72 ± 0.13. (B) Ex4, 12.14 ± 0.06; Ex4(1-30), 11.42 ± 0.03; Ex4(3-39), 8.87 ± 0.06; Ex(9-39) and Ex4(9-30) gave no response (Nasr, Wishart and Donnelly, unpubl.). Note that several peptides [A7-GLP-1, A10-GLP-1, GLP-1(15-36), Ex4(3-39)], believed to be inactive in other expression systems, display robust activity in HEK-293 cells.

of an analogue of Ex4 with enhanced activity (β-Ala replaces Glu-3, while Tyr replaces Gln-3) has shown a longer helical region that extends towards the N-terminus and that may be responsible for its apparent increase in activity (Wang *et al.*, 2010). The NMR structures of GLP-1 and Ex4 are dependent upon the medium in which they reside (Andersen *et al.*, 2002; Neidigh and Andersen, 2002). In the micelle-bound state, the C-terminal extension of Ex4 (residues 31–39) is disordered. However, in aqueous TFE, this region forms a distinct fold called a Tryptophan-cage (Trp-cage), which has been the seed-corn for parallel studies in the field of protein design and folding (e.g. Neidigh *et al.*, 2002). The literature regarding the role of the putative Trp-cage and/or the C-terminal extension of Ex4 has appeared to be contradictory (Doyle *et al.*, 2003; Al-Sabah and Donnelly, 2003a; Runge *et al.*, 2007). However, this has recently been clarified (Mann *et al.*, 2010b) and will be discussed in more detail later in this review.

GLP-1 receptor

The cloning of GLP-1R (Thorens, 1992; Dillon *et al.*, 1993; Graziano *et al.*, 1993; Thorens *et al.*, 1993) identified it as part of the Family B GPCR family (Segre and Goldring, 1993) and hence intimated that its structure would resemble the two-domain fold common to the sub-group. As with many integral membrane proteins, the experimentally determined structure of a representative Family B GPCR has yet to be achieved, although some progress has been made in expressing, purifying and characterizing the full-length hGLP-1R in an *Escherichia coli* expression system (Schröder-Tittmann *et al.*, 2010). Based upon its amino acid sequence, GLP-1R is expected to have the seven transmembrane (7TM) bundle typical of all GPCRs (e.g. Palczewski *et al.*, 2000), with the seven α-helices (TM1-TM7) separated by three intracellular loops (ICL1–ICL3) and three extracellular loops (ECL1–ECL3). However, the sequence of Family B GPCRs is devoid of the consensus sequences and motifs that define Family A receptors, with the possible exception of the disulphide bond between ECL1 and ECL2 (Mann *et al.*, 2010a). However, using approaches that were shown to be reasonably successful at predicting the helical arrangements in Family A GPCRs (Donnelly *et al.*, 1989; 1994; Baldwin, 1993), some studies have attempted to predict the features of the core domain of family B GPCRs (Donnelly, 1997; Tams *et al.*, 1998; Frimurer and Bywater, 1999).

A complicating factor in understanding and determining the structure of family B GPCRs may be their tendency to form oligomers. For example, a predicted lipid-exposed face (Donnelly, 1997) of TM4 of the secretin receptor has been implicated in functionally relevant oligomerization (Harikumar *et al.*, 2007). Disruption of this interface using either peptides derived from the sequence of TM4, or site-directed mutagenesis of Gly^{243} and Ile^{244}, demonstrated that the oligomerization mediated by the lipid-exposed face of TM4 was important for the full potency of secretin. Disruption of the interface resulted in an 18- to 28-fold reduction in potency, despite no effect upon ligand affinity. A similar interface has been identified for the calcitonin receptor (Harikumar *et al.*, 2010).

The cloning of GLP-1R suggested the presence of a signal peptide at the extreme N-terminus, and this was shown to be required for the receptor's synthesis in HEK-293 cells (Huang *et al.*, 2010). Cleavage of the signal peptide was essential for

correct processing and trafficking, such that only the mature and fully glycosylated receptor reached the plasma membrane (Huang *et al*., 2010). The N-glycosylation of GLP-1R had previously been demonstrated in RINm5F cells (Göke *et al*., 1994), with glycopeptidase F reducing the apparent molecular weight from 63 to 51 kDa and tunicamycin reducing detectable receptor expression but not ligand affinity. In recombinant CHO cells, mutation of any two of the three N-glycosylation sites resulted in disruption of receptor trafficking to the plasma membrane (Chen *et al*., 2010a). Therefore, it appears that GLP-1R is a glycoprotein, and that the glycosylation, together with the cleavage of the signal peptide, is an essential process in the correct trafficking and processing of the receptor.

GLP-1R signalling

As with family B GPCRs in general, GLP-1R predominantly signals through the Gs G-protein to raise intracellular cAMP levels (Nauck *et al*., 1993; Coopman *et al*., 2010), although coupling to other G-proteins has been observed (Bahekar *et al*., 2007; Doyle and Egan, 2007). The receptor couples to G-proteins via its intracellular loops, and there is some evidence that demonstrates that different regions and residues couple the receptor to different G-proteins. For example, using synthetic peptides to directly activate G-proteins, Hällbrink *et al*. (2001) have shown that the N-terminal half of ICL3 is responsible for Gs coupling, while the C-terminal half mediates signalling via Gi/o.

Nevertheless, it is the cAMP production mediated by GLP-1R activation that constitutes the principle driver of insulin secretion. Transfection of HIT-T15 cells with GLP-1R enabled GLP-1-mediated insulin secretion in contrast to the transfection of two deletion mutants that were known to be uncoupled from Gs (Salapatek *et al*., 1999). Wheeler's group have scanned the intracellular loops using deletion- and site-directed mutagenesis (Takhar *et al*., 1995; Mathi *et al*., 1997) and have shown that Lys^{334}, along with the two following residues (Leu and Lys), is required for full receptor activity. In addition, Val^{327}, Ile^{328} and Val^{331}, at the TM5/ICL3 junction, play an important part in coupling the receptor and may contact Gs. ECL2 does not play a significant part in G-protein coupling, but mutation of Arg^{176} on ECL1 to Ala reduced receptor activity to about 25% of normal. Heller *et al*. (1996) also identified His^{180} as being important for agonist-induced coupling since its mutation to Arg resulted in a 20-fold decrease in affinity and a 50% decrease in cAMP production. In contrast to Takhar *et al*. (1995), Heller *et al*. (1996) also suggest that Arg^{348} (ECL3) is critical for receptor activity.

Following agonist-induced receptor activation, GLP-1R is internalized. This may be via a coated pit mechanism following phosphorylation by both homologous and heterologous mechanisms (Widmann *et al*., 1995; 1996a). Further investigation located the phosphorylation sites to Ser^{431}/Ser^{432}, Ser^{441}/Ser^{442}, Ser^{444}/Ser^{445} and Ser^{451}/Ser^{452} (Widmann *et al*., 1996b). Alternatively, GLP-1R internalization has been found to be arrestin-independent and associated with caveolin-1 (Syme *et al*., 2006).

N-terminal domain

The two-domain structure of Family B GPCRs lends itself to structural and functional analysis through the isolation of the NTD, an approach that has been extremely useful for studying GLP-1R (Wilmen *et al*., 1996; Xiao *et al*., 2000; Bazarsuren *et al*., 2002; López de Maturana *et al*., 2003; Al-Sabah and Donnelly, 2003a; Mann *et al*., 2007; 2010b; Runge *et al*., 2007; 2008; Underwood *et al*., 2010).

The isolated NTD was expressed and purified from mammalian COS-7 cells and was shown to cross-link to ^{125}I-GLP-1. Using a 6xHis tag, the affinity of the NTD for GLP-1 was estimated to be 450 nM (Xiao *et al*., 2000). Prior to this, Wilmen *et al*. (1996) expressed the isolated NTD of rGLP-1R (residues 20–144) in *E coli* and purified the soluble fraction via a 6xHis tag. The purified protein was expressed at low levels but was shown to be functionally active since it competed with ^{125}I-GLP-1 binding to cells expressing the full-length receptor and could also be cross-linked to ^{125}I-GLP-1 with disuccinimidyl suberate (DSS). Disruption of the disulphide bonds within the NTD using β-mercaptoethanol resulted in its complete loss of ability to compete with ^{125}I-GLP-1 binding to GLP-1R. Bazarsuren *et al*. (2002) also expressed the NTD of hGLP-1R in *E coli*, but, unlike Wilmen *et al*., they isolated the receptor domain from inclusion bodies from which the NTD was denatured, refolded and purified in quantities suitable for biophysical and structural analysis. Interaction with GLP-1 was demonstrated via cross-linking, surface plasmon resonance (SPR) and isothermal titration calorimetry, giving affinity constants for GLP-1 of 46–144 nM. Further analysis demonstrated the presence of three disulphide bonds linking residues 46–71, 62–104 and 85–126. Using this expression/refolding approach, López de Maturana *et al*. (2003) expressed and purified the NTD from rGLP-1R and demonstrated that, while GLP-1 binds with 400 nM affinity, the ligands Ex4, Ex4(3-39) and Ex4(9-39) bind with 1–3 nM affinity. The differential affinity of GLP-1 and Ex4 will be discussed in more detail in the next section.

The isolated NTD of hGLP-1R from the *E coli* inclusion body preparation was biophysically characterized by Runge *et al*. (2007), and the same protocol was used to produce a protein preparation which was incubated with Ex4(9-39), before being further purified, crystallized and analysed using X-ray diffraction (Runge *et al*., 2008). The resultant 2.2Å crystal structure resembled that of other Family B GPCR NTDs, displaying two regions of anti-parallel β-sheet, three disulphide bonds and an N-terminal α-helix (Parthier *et al*., 2009; Figure 3A). The core of the structure contains six conserved residues (Asp^{67}, Trp^{72}, Pro^{86}, Arg^{102}, Gly^{108} and Trp^{110}), which are critical for the fold's stability and structure. A ligand-binding groove is present, which is lined by residues on the α-helix, turn 1, loop 2 and the C-terminal region of the NTD.

This inclusion body/refolding approach has been extended to include the full-length receptor that has been obtained in an active form with distinct binding kinetics from those of the autonomously folded NTD (Schröder-Tittmann *et al*., 2010). However, to date, the structure of the full-length receptor remains unknown. An attempt was made to model GLP-1R and its ligand-binding sites using homology modelling with the templates being the crystal structures of the GIP receptor NTD and rhodopsin (Lin and Wang, 2009). More recent modelling (Miller *et al*., 2011) has utilized the X-ray structure of the GLP-1R NTD (Underwood *et al*., 2010) fused to 250 diverse helical bundle domain models from 25

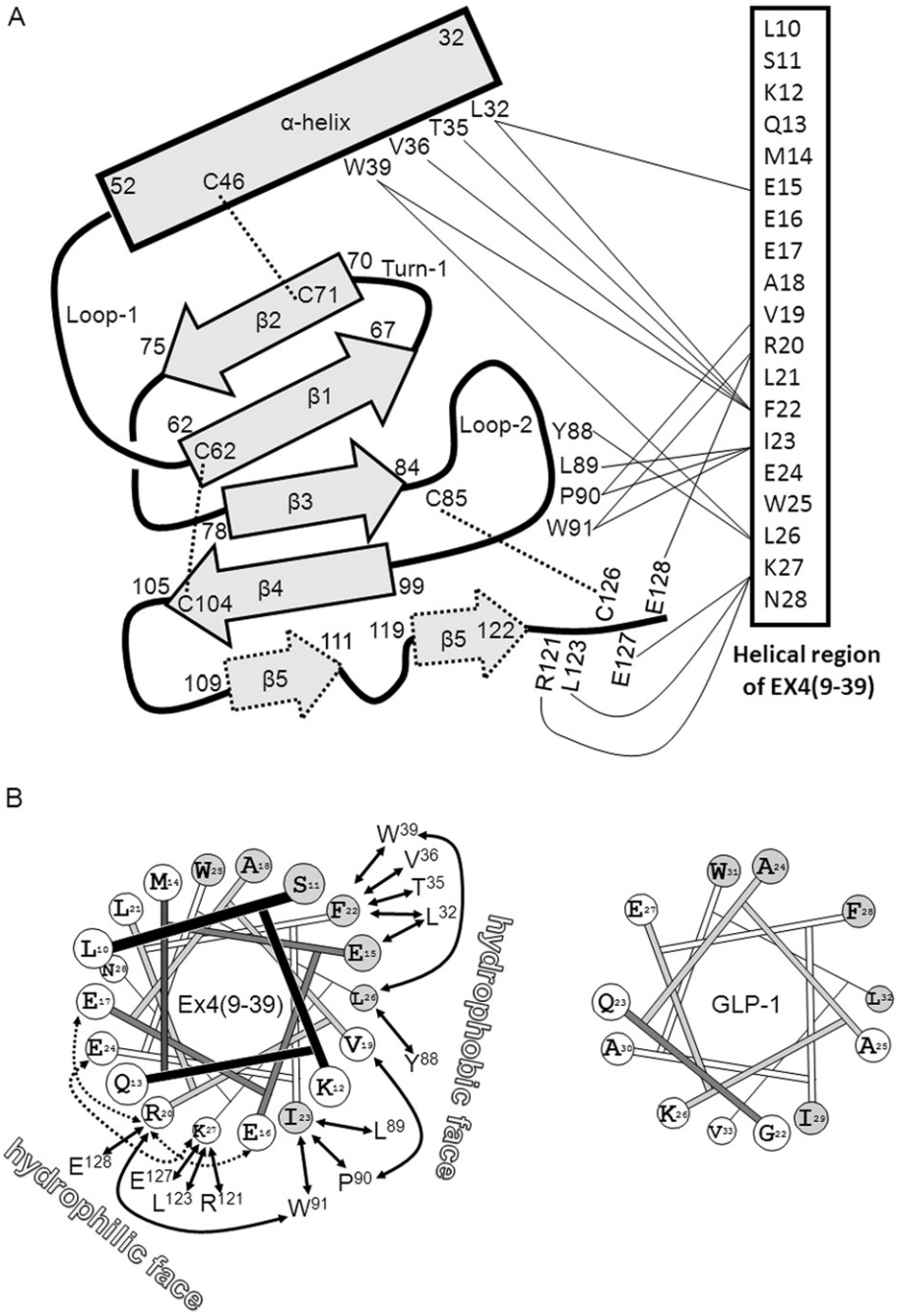

Figure 3

(A) A schematic representation of the structure of the NTD of hGLP-1R based upon the crystal structure of Runge *et al.* (2008). The residue numbers at the boundaries of the various secondary structural elements are shown. Disulphide bonds are depicted with dashed lines. On the right is the α-helical region of Ex4(9-39) with lines connecting to contacting residues on the NTD. (B) *Left*: Ideal helical wheel containing the residues in the helical region of Ex4(9-39). Residues conserved with GLP-1 are filled grey. Interactions with residues in the NTD are shown with solid arrows. Intra-helical interactions, which stabilize the helix, are shown with dotted arrows. The hydrophilic and hydrophobic faces are labelled. *Right*: The helical region of GLP-1 on the C-terminal side of the Gly-22 kink. Note that the helix-stabilizing interactions found in Ex4(9-39) are not formed in GLP-1 since the residues are not conserved.

transmembrane bundles, alongside experimentally derived photo-affinity contacts, in order to further understand the ligand–receptor interactions. Nevertheless, while it appears reasonable to assume that the core domain will interface with the NTD in the region close to the N-terminal ends of the helices of both the NTD and the ligand, the number of degrees of freedom remains very high and a structure of the full-length receptor will be required before we can fully

understand the details of this interface. Further complexities have been proposed by a mutational analysis of the NTD of the related glucagon receptor using cysteine accessibility approaches (Prévost *et al.*, 2010). A number of residues on one face of the NTD were found to be much less accessible to covalent modification than would have been expected from the GLP-1R NTD structures. However, these residues are on the opposite face of the NTD relative to the expected interface of the core domain and therefore may form an interface with another, as yet unidentified, protein.

Peptide–receptor interactions

Stage 1 interaction – binding of the ligand to the NTD

As described previously, the isolation of the NTD of GLP-1R as an independent soluble domain resulted in specific, though low affinity, binding of the natural ligand GLP-1. Since several mutations in the core domain of full-length GLP-1R resulted in a lowering of GLP-1 affinity, but not that of Ex4, it was hypothesized that Ex4 affinity was largely independent of the core domain (López de Maturana and Donnelly, 2002; Al-Sabah and Donnelly, 2003b). This was shown to be the case by demonstrating that the complete removal of the core domain has a minimal effect upon Ex4 affinity, compared with that of GLP-1, and that the interaction between Ex4 and the NTD was not dependent upon the N-terminal region of the ligand (López de Maturana *et al.*, 2003). A model was proposed to explain the distinct pharmacological properties of the two peptide agonists at the isolated NTD, with the suggestion that Ex4 formed an additional interaction (termed the 'Ex' interaction), which was due to one or more of the unique structural features of Ex4, such as its increased helicity, its C-terminal extension, or residues that were not conserved with GLP-1 (López de Maturana *et al.*, 2003). Runge *et al.* (2007) demonstrated that it was the increased helicity of Ex4, which accounted for its high affinity at the fully isolated NTD. However, when this domain was fused to the plasma membrane of HEK-293 cells via TM1, the large differential affinity observed at the isolated NTD of hGLP-1R was reduced to only eightfold (Mann *et al.*, 2010a,b) and, furthermore, in the presence of Brij micelles, the affinity of GLP-1 and Ex4 at the isolated NTD was similar (Schröder-Tittmann *et al.*, 2010).

The nine-residue C-terminal extension of Ex4 plays no significant role in the peptide's affinity at hGLP-1R (Runge *et al.*, 2007; Mann *et al.*, 2010a,b), which is in agreement with the X-ray structure of the isolated NTD bound with Ex4(9-39) (Runge *et al.*, 2008). However, Ser^{32} in this extended region of the peptide is able to interact with Asp^{68} in the NTD of rGLP-1R, generating a >20-fold improvement in affinity (Mann *et al.*, 2010a,b). The improved affinity of Ex4(9-39) at rGLP-1R, resulting from interaction between Ser^{32} of the ligand and Asp^{68} of the NTD, could be exchanged between hGLP-1 and rGLP-1R by swapping Glu and Asp at position 68. Hence, despite sharing similar physiochemical properties to Asp, Glu-68 does not appear to interact strongly with Ser^{32} of the ligand. Given that Glu-68 interacts with the C-terminus of GLP-1 (Day *et al.*, 2010), it is possible that it preferentially interacts with residue 30 of Ex4(9-39), while the shorter Asp^{68} side chain in rGLP-1 interacts with Ser^{32}. This would explain why the C-terminal extension on Ex4(9-39) improves the affinity for rGLP-1R but not hGLP-1R.

Photo-crosslinking of the C-terminal region of GLP-1 to GLP-1R demonstrated that residue 35 interacts with Glu-125, while residue 24 interacts with Glu-133 (Chen *et al.*, 2009). On the other hand, Ex4(9-39) retains high affinity for the isolated rGLP-1R NTD consisting of residues Gln-26–Glu-127, suggesting that the NTD from Glu-128 onwards is not required for binding the C-terminal half of the ligand (Mann *et al.*, 2007). The X-ray crystal structure of the hGLP-1R fragment Arg^{24}–Tyr^{145}, with either Ex4(9-39) (Runge *et al.*, 2008) or GLP-1 (Underwood *et al.*, 2010), reveal the details of the interaction of the C-terminal region of the peptides and the NTD of the receptor. Figure 3A summarizes the structure of the NTD and how it binds to Ex4(9-39). The antagonist forms a well-defined helix from residues Leu^{10}–Asn^{28} and interacts with the NTD using residues within Glu^{15}–Ser^{32}. Although residues 9–14 do not interact with the NTD, residues 10–14 are nevertheless critical for high affinity binding (Runge *et al.*, 2007), presumably because they are required to stabilize the helical structure of the ligand. The α-helix of Ex4(9-39) is amphipathic (Figure 3B) and interacts with the NTD via residues on both faces, although it is the hydrophobic face which appears most important, with Val^{19}, Phe^{22}, Ile^{23} and Leu^{26} being buried in the binding site.

GLP-1 also forms an α-helix when bound to the NTD (Underwood *et al.*, 2010), but, in contrast to that in Ex4(9-39), it is kinked around Gly^{22} as observed in earlier NMR studies (Thornton and Gorenstein, 1994). Residues Ala^{24}–Val^{33} interact with the NTD and, as with Ex4(9-39), the principal contacts are via a hydrophobic interface formed by residues Ala^{24}, Ala^{25}, Phe^{28}, Ile^{29}, Leu^{32} and Val^{33}. Because the hydrophobic faces of the helices of Ex4(9-39) and GLP-1 are highly conserved (López de Maturana and Donnelly, 2002), their interactions with the NTD are very similar.

However, differences at the hydrophilic faces result in a degree of differential affinity. The ability of Arg^{20} of Ex4(9-39) to form intra-helical interactions with Glu^{16} and Glu^{17}, and also for Lys^{27} to interact intra-helically with Glu^{24}, results in stabilization of the α-helix (Figure 3B). However, three of these four charged sites are absent in GLP-1, and, moreover, Glu^{16} is replaced by Gly^{22}, which reduces the helix stability and enables the distortion of this region. This hydrophilic surface may also be involved in a direct receptor interaction, since the crystal structures suggests that Arg^{20} [Ex4(9-39)] and Lys^{26} (GLP-1) may interact with Glu^{128} of the NTD. However, truncation of the NTD to remove Glu^{128} did not remove high Ex4(9-39) affinity (Mann *et al.*, 2007). On the other hand, Glu^{127}, which appears to interact with the antagonist but not GLP-1, could not be truncated without loss of Ex4(9-39) affinity, suggesting that the observed interaction with Lys^{27} of the antagonist is important (Mann *et al.*, 2007). Mutagenesis of Glu^{127} resulted in a sevenfold reduction of Ex4(9-39) affinity but with no effect upon GLP-1, suggesting it may be responsible for the differential affinity of these peptides at the NTD (Underwood *et al.*, 2010). Given that their differential affinity is about eightfold at the membrane-tethered NTD (Mann *et al.*, 2010b), the specific Glu^{127} interaction may indeed account for this. In addition, the mutation of Leu^{32}, previously identified as a potential binding partner of Ex4(9-39)

(Mann *et al.*, 2007), also resulted in specific reduction of the antagonist's affinity, although the explanation for this is not obvious from the crystal structures (Underwood *et al.*, 2010). It may be that, since Leu[32] interacts with the ligand's helix close to the point at which GLP-1 kinks, it may have a differential interaction with the two ligands. Stabilization of the helix in this region of GLP-1, by substituting Ser[18] by Lys and Gly[22] by Glu, resulted in an eightfold increase in potency (Murage *et al.*, 2008; 2010).

Further studies have shown important ligand–receptor interactions points on the NTD. A polymorphism of GLP-1R, Thr[149]–Met (Beinborn *et al.*, 2005), resulted in a 60-fold reduction in affinity for GLP-1 but only fivefold reduction for Ex4, suggesting that this residue is involved in GLP-1 binding. However, the lack of a major effect on Ex4 affinity suggests that, either it does not interact with Thr[149], or else it interacts with a region not essential for affinity, such as the N-terminus. The latter seems likely since the close-by residue Tyr[145] has been labelled by a peptide probe containing benzoylphenylalanine (BPh) in place of Thr[12] of GLP-1 (Chen *et al.*, 2010b), and, furthermore, a similar ligand probe with BPh replacing Val[16] was found to cross-link to Leu[141] (Miller *et al.*, 2011). The importance of helix stability in this region has been discussed by Neumann *et al.* (2008), and, indeed, Thr[12] and Val[16] would be predicted to be separated by one helical turn. These data therefore complement the study of Murage *et al.* (2008), who demonstrated that stabilization of helical structure between Thr[11] and Gly[22], using lactam bridges, improved activity. Therefore, the region linking the NTD to TM1 appears to interact with the N-terminal region of GLP-1, although, as would be expected if this was the case, this region is not required for the high affinity of Ex4(9-39) since the NTD can be truncated between Glu[127] and Glu[128] (Mann *et al.*, 2007).

Stage 2 interaction – binding of the ligand to the core domain

Since there is no determined structure for the core domain of GLP-1R, our knowledge of the interaction between the peptide ligands and the core domain must be gleaned from more indirect approaches. The identification of mutations which reduce the affinity of GLP-1, but not that of GLP-1(15-36), have been useful for identifying residues in the core domain that interact with the N-terminus of GLP-1. For example, a double-alanine mutagenesis scan of ECL1 of rGLP-1R identified a residue pair Met[204]–Tyr[205] as interacting with GLP-1's N-terminal 8 residues (López de Maturana *et al.*, 2004). This approach has been validated by the identification of Tyr[205] as the attachment point of a photoactive analogue of GLP-1 containing a BPh at the N-terminus (Chen *et al.*, 2010b). While the scan of ECL1 suggested that it did not play a major role in ligand recognition (López de Maturana *et al.*, 2004), a number of charged and polar residues have been identified as being involved in ligand binding (Xiao *et al.*, 2000).

Further mutations with differential effects upon GLP-1 and GLP-1(15-36) affinity have been used to identify Asp[198] (TM2/ECL1; López de Maturana and Donnelly, 2002) and Lys[288] (TM4/ECL2; Al-Sabah and Donnelly, 2003b) as interaction points for the N-terminus of GLP-1. Furthermore, a double Ala-scan of ECL2 also identified a number of sites likely to be important in forming the binding site of the peptide's N-terminus (Table 1) and are also likely to be important for the agonist-induced activation of the receptor (Mann *et al.*, 2010a,b). However, ECL2 may also contact the mid-region of the peptide since BPh at position 20 of GLP-1 was photo-crosslinked to Trp[297] (Miller *et al.*, 2011). The use of GLP-1/glucagon chimaeric ligands, coupled with site-directed mutagenesis of the glucagon receptor, has shown that the reduced activity of an analogue of glucagon, with Ala at position 2, could be rescued by mutating Asp[385] to Glu, suggesting that the N-terminus binds close to the TM7/ECL3 boundary (Runge *et al.* (2003a,b).

A molecular modelling study, which incorporated the NTD X-ray studies (Runge *et al.*, 2008; Underwood *et al.*, 2010) with the outcome of several photo-crosslinking studies (Miller *et al.*, 2011; Chen *et al.*, 2009; Chen *et al.*, 2010b), predicted a deep binding pocket for His[7] of GLP-1, with Thr[11] close to the receptor sites Tyr[145] and Tyr[205]. Meanwhile Lys[26] and Leu[20] of GLP-1 were placed close to Glu[133] and Tyr[297] respectively (Miller *et al.*, 2011). However, without knowledge of the structures of the GLP-1R core domain structure and the receptor-bound ligand, it remains difficult to fully understand the details of the stage 2 interaction.

Table 1

pIC_{50} values for GLP-1 and GLP-1(15-36) at wild-type rGLP-1R expressed in HEK-293 cells and at site-directed mutations targeted at pairs of residues in ECL2 (Al-Sabah and Donnelly, unpublished)

	GLP-1(7-36) pIC_{50}	GLP-1(15-36) pIC_{50}
GLP-1R	8.5 ± 0.07	6.4 ± 0.02
W297A-T298A	6.5 ± 0.06	6.7 ± 0.27
R299A-N300A	6.1 ± 0.06	6.3 ± 0.06
Y305A-W306A	6.0 ± 0.13	6.2 ± 0.05
L307A-I308A	6.1 ± 0.09	6.0 ± 0.05
I309A-R310A	6.8 ± 0.08	6.5 ± 0.02

pIC_{50} values were calculated from heterologous competition binding assays using ^{125}I-Ex4(9-39) tracer. Methodology, cell lines and reagents are as described in Al-Sabah and Donnelly (2003b). The mean ± SE is shown for three independent experiments each performed in triplicate and shows that the mutations remove the sensitivity of the receptor to the presence of the N-terminal region of GLP-1.

Non-peptide ligands

Non-peptidic agonists of GLP-1R would be desirable as therapeutic agents since they have the potential to be orally active and hence circumvent the current requirement for self-injection by patients. However, the first non-peptide ligand at GLP-1R was in fact an antagonist, T-0632, which competes with ^{125}I-Ex4(9-39) at hGLP-1R with an IC_{50} of 1.2 μM and was able to antagonize GLP-1-induced cAMP production in COS-7 cells (Tibaduiza *et al.*, 2001). The compound was significantly less potent at rGLP-1R, and this species difference enabled the

identification of Trp^{33} (Ser^{33} in rGLP-1R) as being the critical residue. This residue is located on the α-helix of the NTD but is not involved in peptide binding – presumably the non-peptidic binding site over-laps with that of the peptide.

The first two non-peptide agonists of GLP-1R were published in 2007 (Chen *et al.*, 2007; Knudsen *et al.*, 2007). Boc5 and its analogue S4P were discovered through a screen of more than 48 000 synthetic and natural compounds that yielded two hits, which actually turned out to be due to the dimeric versions of the original compounds that had formed spontaneously during their long-term storage in DMSO. Boc5 acted as a full agonist with 1.08 μM potency at rGLP-1R expressed in HEK-293 cells using a Luciferase-based cAMP reporter assay. Ex4(9-39) blocked the Boc5-induced receptor response and also competed with ^{125}I-GLP-1 binding. Furthermore, Boc5 elicited a glucose-induced insulin response in isolated pancreatic islets from rat, inhibited food intake in rats and reduced HbA1c levels in *db/db* mice to non-diabetic levels. The GLP-1 mimetic properties of Boc5 were further characterized by Su *et al*. (2008).

The other class of non-peptidic agonist discovered in 2007 was 'Compound 2' (Cmp2; 6,7-dichloro-2methylsulfonyl-3-*N*-*tert*-butylaminoquinoxaline) and related structures (Knudsen *et al.*, 2007; Teng *et al.*, 2007). These compounds are significantly smaller than Boc5 and have distinct pharmacological properties. They are ago-allosteric modulators of hGLP-1R, since they can act both as independent agonists but also as allosteric enhancers of the activity of the natural agonist. In this case, the allosteric enhancement initially appeared to be limited to an increase in GLP-1 affinity (26-fold) rather than potency, probably due to a high degree of spare receptors. However, Cmp2 did potentiate the potency of GLP-1 in stable FlpIn-CHO cells expressing hGLP-1R (Koole *et al.*, 2010).

The concentration–response curves for Cmp2 are bell-shaped since higher concentrations are associated with cell death (Coopman *et al.*, 2010). Nevertheless, Cmp2 was able to stimulate glucose-dependent insulin secretion from normal mouse islets but not from those derived from GLP-1R knock-out mice (Knudsen *et al.*, 2007). Related quinoxaline compounds have also been synthesized and shown to display anti-diabetic activity (Bahekar *et al.*, 2007). A comparison of the effects of Cmp2 and GLP-1 in HEK-293 cells expressing hGLP-1R demonstrated that both agonists act via Gαs and that Cmp2 elicits 88% of the E_{max} of GLP-1 in cAMP assays (Coopman *et al.*, 2010). Interestingly, Cmp2's cAMP response was enhanced by Ex4(9-39), and, furthermore, the antagonist inhibited Cmp2-mediated receptor internalization. Further *in vivo* studies using mice demonstrated that, while Cmp2 failed to prominently enhance glucose-mediated insulin release when compared with the more potent peptidic drugs exenatide and liraglutide, it did nevertheless significantly decrease the overall glucose excursion (Irwin *et al.*, 2010). Ex4(9-39) did not impair Cmp2's glucoregulatory actions, consistent with this antagonist's inability to block Cmp2-induced cAMP activity *in vitro* (Knudsen *et al.*, 2007; Coopman *et al.*, 2010). Cmp2 also appears to possess peptide-dependent allosteric properties since it displayed clear enhancement of potency and affinity at GLP-1R for oxyntomodulin but not Ex4 (Koole *et al.*, 2010).

An interesting non-peptide response at GLP-1R has been shown using the flavanoid quercetin which, despite not being able to activate GLP-R directly, enhanced the potency of the Ca^{2+} response mediated by GLP-1 and Ex4 (Koole *et al.*, 2010). Using a range of flavanoid structures, the SAR between this pathway-selective behaviour and the flavanoid structure identified the 3-OH group as the key moiety of this activity (Wootten *et al.*, 2011).

The pharmacology of a novel pyrimidine-based compound has recently been published by Sloop *et al*. (2010). 'Compound B' (CmpB) was discovered from screening a pharmacophore-based focused compound library, followed by rational modifications of the initial hit. It is a full agonist at hGLP-1R but displayed poor potency (0.7 μM) that is more than 1000-fold lower than GLP-1 itself. Nevertheless, CmpB evoked insulin secretion in rats and was able to normalize insulin secretion in islets derived from a human donor with type 2 diabetes. CmpB was able to activate a truncated version of GLP-1R, which lacks the NTD, demonstrating that this non-peptide acts via the core domain. Ex4(9-39) did not antagonize the CmpB-response, further demonstrating that its mode of binding is distinct from that of the peptide ligands.

Conclusion

The case for targeting GLP-1R for the treatment of type 2 diabetes is clear, and therefore, knowledge of the details of the peptide ligands, the receptor itself and their mode of interaction is important. Much is now known about the structure and function of GLP-1R and of its natural ligand GLP-1, while some initial progress has been made in the discovery of non-peptidic agonists. Many of the key recognition elements of the peptide are known and the sites to which they bind on the receptor have been partly deduced. Nevertheless, a great deal of work has yet to be carried out before our understanding of this important hormone–receptor interaction is complete and before the use of non-peptidic therapeutics becomes a reality.

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Conflict of interest

The author declares no conflict of interest.

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