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The GLP-1 receptor and its peptide ligands.

Glucagon-like peptide 1 (7-36) amide (GLP-1) is a 30 amino acid peptide produced in intestinal L cells and released into the bloodstream in response to food intake. It is a potent ‘incretin’, in that it increases glucose-dependent secretion of insulin by pancreatic β-cells. GLP-1 has also been shown to stimulate pro-insulin gene transcription in the pancreatic β-cells, slow down gastric emptying time and reduce food intake. As a result of these actions, GLP-1 has received much attention as a possible therapeutic agent in the treatment of type II diabetes and obesity. Unlike the sulphonylureas, the actions of GLP-1 are glucose-dependent and do not produce hypoglycemic side effects. However, GLP-1 is rapidly degraded in vivo by dipeptidyl peptidase IV (DPP IV), and has a half-life of 2 min. Hence, future therapeutic agents would have to be physiologically more stable than GLP-1.

Exendin-4, found in the venom of the Gila Monster (*Heloderma suspectum*), is also an agonist for the GLP-1 receptor (GLP-1R). It is resistant to DPP IV digestion and, unlike GLP-1, it can be truncated by 8 amino acid residues at its N-terminus without losing receptor affinity. However, the loss of the first 2-8 amino acid residues results in the generation of antagonists. The N-terminal region of GLP-1 and exendin-4 are almost identical, a significant difference being the second amino acid residue, alanine in GLP-1 and glycine in exendin-4, which gives exendin-4 its resistance to DPP IV digestion. Exendin-4 has an extra 9 amino acid residues at its C-terminus which have been shown to form a ‘Trp-cage’ by NMR. NMR analysis of exendin-4 also shows that the central region (amino acid residues 10-30) is helical in structure. Interestingly GLP-1 and Exendin 4 only share 8 amino acid residues in this region but since they lie on the same face of the α-helix, we postulate that this face of the helix interacts with the receptor.

The GLP-1 receptor (GLP-1R) has been cloned and is a member of the ‘family B’ G protein-coupled receptors (GPCRs). Other members of this family include receptors for glucagon, calcitonin, glucose-dependent insulinotropic polypeptide and vasoactive intestinal peptide. It is known that the large amino terminal domain that characterizes the ‘family B’ GPCRs plays a key role in ligand binding. However the amino terminus is not entirely sufficient to bind the ligand and regions in the extracellular loops and/or transmembrane helices are also believed to provide additional interactions.

Which regions of exendin-4 contribute to its unique properties?
The aim of this study was to build on the binding site model previously proposed by our laboratory by identifying the regions of exendin-4 that contribute to its ability, relative to GLP-1, to maintain high affinity binding following truncation of its N-terminal residues. This, we hope, will lead us to design more potent GLP-1R agonists and antagonists.

For the purpose of this study, radioligand binding assays were carried out on the wild type rat GLP-1 receptor (rGLP-1R) and a truncated form of the receptor consisting of the N-terminal domain and the first transmembrane helix (rNT-TM1). These receptors were expressed in HEK-293 cells, and binding assays were carried out on membrane preparations. Competition binding assays were carried out using $^{125}$I-exendin-4(9-39) and six unlabelled peptide ligands (see Fig.
The six peptides were chosen in order to determine the relative contribution to affinity for the N-terminal region, central helical region and, in the case of exendin-4, the C-terminal “Trp-cage”. These peptides are drawn schematically in Fig. 1B.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>rGLP-1R</th>
<th>rNT-TM1</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLP-1 (1-30)</td>
<td>7.8 ± 0.10</td>
<td>6.1 ± 0.11</td>
</tr>
<tr>
<td>GLP-1 (9-30)</td>
<td>6.4 ± 0.03</td>
<td>6.3 ± 0.08</td>
</tr>
<tr>
<td>exendin-4 (1-39)</td>
<td>8.5 ± 0.12</td>
<td>7.9 ± 0.13</td>
</tr>
<tr>
<td>exendin-4 (9-39)</td>
<td>8.1 ± 0.07</td>
<td>7.9 ± 0.11</td>
</tr>
<tr>
<td>exendin-4 (1-30)</td>
<td>7.1 ± 0.11</td>
<td>6.5 ± 0.11</td>
</tr>
<tr>
<td>exendin-4 (9-30)</td>
<td>6.7 ± 0.06</td>
<td>6.8 ± 0.13</td>
</tr>
</tbody>
</table>

Table 1. pIC$_{50}$ values (M) from competition binding assays using radiolabelled antagonist $^{125}$I-Exendin (9-39). The data represent the mean and S.D. for three independent experiments.

As expected, N-terminal truncation of the GLP-1 peptide resulted in a large loss in affinity (25-fold) while the equivalent truncation of exendin-4 to exendin-4 (9-39) resulted in only a small reduction (2.5-fold). The latter observation can also be confirmed by the 2.5-fold reduction in affinity between exendin-4 (1-30) and exendin-4 (9-30). The reduced contribution to affinity by the N-terminal region of exendin-4 is due to the substitution of Ala-2 for Gly. Although this reduces affinity, it actually enhances its resistance to DPP IV and hence actually increases its potency in vivo. However, if the N-terminal region of exendin-4 contributes less to affinity than that of GLP-1, how is it that the affinity of exendin-4 is higher than that of GLP-1?

Firstly, it can be seen that the affinity of the core helical region (9-30) of exendin-4 contributes more to affinity than that of GLP-1. In addition, the C-terminal region of exendin-4 enhances its affinity further. The removal of the C-terminal region of exendin-4, yielding exendin-4 (1-30), resulted in a 25-fold reduction in its affinity. This is also confirmed by removing the C-terminal region from exendin-4 (9-39) to yield exendin-4 (9-30). Hence, we can conclude that while the affinity of the core helical region of exendin-4 is only enhanced 2.5 fold by its N-terminus, the addition of the C-terminal region enhances its affinity a further 25-fold. Hence this peptide maintains high affinity as well as DPP IV resistance.

The analysis of the rNT-TM1 receptor highlights the receptor domains to which the three regions of the peptides bind. The removal of the N-terminal regions of the peptides results in no loss of affinity at rNT-TM1, suggesting that this receptor domain is not involved in binding the N-termini of the peptides. This confirms our earlier studies. However, the central helical regions of the peptides maintain affinity for the N-terminal domain of the receptor, suggesting that they do not require sites on the transmembrane and extracellular loop regions of the receptor. The C-terminal “Trp cage” of exendin-4 is essential for high affinity binding to both the full length and truncated receptors. Hence, this region clearly binds to the N-terminal domain of the receptor.

**A model for peptide:receptor binding.**

These studies provide further evidence for our published model of how GLP-1R binds peptides. In this model, we defined three interactions “N”, “H” and “Ex”. Fig. 1C shows which of the three interactions are present with each peptide : receptor combination and it can be seen that this is entirely compatible with the binding data shown above. In previous and on-going work, we have identified several specific residues involved in the “N” interaction. In the work described here, we have identified the major components of the “Ex” interaction (the interaction that enhances exendin-4 affinity over that of GLP-1 and removes the dependence of high affinity
upon the N-terminal region) as being the small additional contribution from the helical core domain and a much larger contribution from the C-terminal “Trp Cage”.

**Publications**


**Acknowledgements**

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