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Characterization of the Binding of [^{125}I]GLP-1(9-36) amide, the Major Metabolite of the Insulin Secretagogue, Glucagon-like peptide 1 (GLP-1) and Function of the Unlabelled Peptide in Murine Aorta

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Glucagon-like peptide 1 (GLP-1) is derived from the transcription product of pro-glucagon genesis, synthesized in the intestine and released in response to meal ingestion and efficiently lowers blood glucose in Type 2 diabetic patients. The GLP-1R (glucagon-like peptide-1 receptor) controls the physiological response to GLP-1 and is a major target for the development of drugs owing to the broad range of potential beneficial effects in Type 2 diabetes. GLP-1(7-36) is the biologically active form and has a very short half-life. It is rapidly metabolized by dipeptidyl peptidase IV (DPP-4) to the major metabolite GLP-1(9-36)-amide, which comprises about 60% of circulating GLP peptides. Inhibitors of this enzyme are widely used to treat diabetes. GLP-1(9-36) is often though to be inactive with only low affinity for the GLP-1 receptor. However acute dosing of GLP-1 (9-36) potently inhibits hepatic glucose production in obese mice with a similar effect in lean mice in the presence of GLP-1 antagonist, exendin (9-39) (Elahi et al., 2008). This anti-diabetogenic action of GLP-1(9-36) may have therapeutic benefit.

However, it is unclear whether GLP-1(9-36) mediates functional activity via specific receptors. Our aim was to characterise the binding of the novel ligand [^{125}I]GLP-1(9-36) in tissues from mouse and measure pharmacodynamic parameters compared with [^{125}I]GLP-1(7-36). Secondly, to compare the binding of GLP-1(7-36) and GLP-1(9-36) in cell lines artificially expressing GLP1-R and finally, to compare functional responses of both peptides in mouse aorta.

Adult mice, C57/BL6J (males and females, 25-35g); were killed with CO_2 and brain and aorta removed prior to binding and functional experiments. Following pre-incubation of fresh frozen section of cryostat sections (Bregma~-0.7-1.0) from adult mouse brains, binding assays (Maguire et al.,2012) were carried out using increasing concentration of either [^{125}I]GLP-1(7-36) or [^{125}I]GLP-1(9-36) for 90 min at room temperature. Non-specific binding was defined by 10 μM of the GLP-1(7-36) and GLP-1(9-36). In functional experiments, mouse aortae were mounted in wire myographs and the effect of increasing concentrations of each peptide measured. Binding of peptides to recombinant GLP-1 receptors was measured using cAMP and scintillation proximity assays.

Table 1 Characterization of peptides in native tissue and recombinant GLP-1 receptors

Ligand binding (mouse brain, n=4)	K_D (nM)	B_{MAX} (fmol/mg)	nH
[^{125}I]GLP-1(7-36)	1.29 ± 0.26	57.0 ± 14.5	0.96 ± 0.06

[^{125}I]GLP-1(9-36)	0.214 ± 0.08	2.69 ± 0.74	1.06 ± 0.05
Vasoconstrictor Assay (mouse aorta, n=3)	pD2 (nM)	E_{MAX} %KCl maximum	
GLP-1(7-36)	7.69±0.24	35±8%	-
GLP-1(9-36)	7.57±0.64	25±7%	-

In the mouse brain both labelled peptides bound with a single high sub-nanomolar affinity, with Hill slopes close to unity. The density of receptors was an order of magnitude lower for [^{125}I]GLP-1(9-36). In functional experiments both peptides had similar potencies. These results suggest GLP-1(9-36) has functional activity distinct from the GLP-1R but the precise molecular mechanism is unclear.

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

Maguire JJ, Kuc RE, Davenport AP. Radioligand binding assays and their analysis. Methods Mol Bio; 897:31-77, 2012.