Glucagon-like peptide-1 does not have a role in hepatic carbohydrate metabolism

M. Ghiglion¹, E. Blazquez², L. O. Uttenthal¹, J. G. de Diego², E. Alvarez², S. K. George¹ and S. R. Bloom¹

¹Department of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London, UK and

²Departamento de Fisiologia, Facultad de Medicina, Universidad de Salamanca, Salamanca, Spain

Summary. Glucagon-like peptide-1 does not have specific, high-affinity receptors on rat liver membranes, does not displace glucagon from glucagon receptors on these membranes and does not stimulate the production of cyclic AMP by isolated rat hepatocytes. In the presence of glucagon, high concentrations of glucagon-like peptide-1 do not significantly al-

ter the production of cyclic AMP. Thus, glucagon-like peptide-1 appears unlikely to have a direct action on hepatic carbohydrate metabolism.

Key words: Glucagon-like peptide-1, glucagon, specific receptors, cyclic AMP, liver cells.

Glucagon-like peptide-1 (GLP-1) is one of two glucagon-like peptide sequences present in the C-terminal region of the deduced sequence of mammalian pre-pro-glucagon [1]. Its amino-acid sequence, completely conserved between hamster [1], man [2], ox [3] and rat [4], presents a homology of 14 out of the 29 residues comparable with glucagon. It is present in extracts of human pancreas, although larger molecular forms predominate, and in glucagon-producing pancreatic tumours [5]. We have recently shown that GLP-1 does not have a glucagon-like effect on plasma glucose in cortisone-pretreated rabbits [6], whilst others have demonstrated that GLP-1-(1-36)-peptide amide and human GLP-2 activate rat hypothalamic and pituitary adenylate cyclase [7]. In order to investigate further a possible role of GLP-1 in glucose metabolism, we have looked for evidence of specific, high-affinity GLP-1 receptors on rat liver membranes. We have also investigated whether GLP-1 was capable of stimulating cyclic AMP production in isolated rat hepatocytes. As the GLP-1 sequence ends with a glycine residue as a possible donor for C-terminal amidation [8], both synthetic GLP-1-(1-37) and synthetic GLP-1-(1-36)-peptide amide were studied.

Materials and methods

Synthetic GLP-1-(1-36)-peptide amide was purchased from Peninsula Laboratories Inc., Belmont, CA, USA, and synthetic GLP-1-(1-37) from Bachem, Torrance, CA, USA. ¹²⁵I-GLP-1-(1-37) was prepared by iodination with carrier-free Na¹²⁵I (IMS.30, Amersham International, Amersham, UK) using the chloramine-T method [9]. The iodinated peptide was separated from both unreacted iodine and unlabelled peptide by reverse-phase high pressure liquid chromatography on a Techsil 5 µm particle size octadecylsilylsilica column eluted with acetonitrile: water: trifluoroacetic acid 450:550:1 by volume. The labelled GLP-1-(1-37) showed a specific activity of 26 Bq/fmol.

The preparation of mono-¹²⁵I-glucagon, partially purified rat liver membranes and isolated rat hepatocytes, as well as the measurement of peptide binding to liver membranes and the production of cyclic AMP by rat hepatocytes, were carried out as described previously [10].

For the measurement of peptide binding to liver membranes, the membranes (4150–4850 µg protein per ml) were incubated in Krebs Ringer phosphate buffer [10], pH 7.5, containing 10 g/l bovine serum albumin (BSA) with different concentrations of unlabelled glucagon, GLP-1-(1-37) or GLP-1-(1-36)-peptide amide and a constant concentration of mono-¹²⁵I-glucagon (0.188 pmol/ml) or ¹²⁵I-GLP-1-(1-37) (0.85 pmol/ml) for 4 h at 10 °C. These conditions have been shown to give optimal specific ¹²⁵I-glucagon binding [10]. At the end of the incubation period, the samples (total volume 250 µl) were diluted out rapidly with 1 ml of ice-chilled buffer and immediately filtered on Oxoid filters (0.45 µm pore-size) soaked in Krebs Ringer phosphate buffer containing 100 g/l BSA for at least 1 h before use. Non-specific adsorption of ¹²⁵I-glucagon to the membranes was determined in the presence of 1×10^{-6} mol/l unlabelled glucagon.

The isolated hepatocytes were prepared from 48-h-fasted male Wistar rats by collagenase perfusion [10]. The cells (7.5×10^6 /ml) were incubated at 37 °C for 2.5 min with Krebs Ringer bicarbonate [10], pH 7.4, containing 10 mmol/l alanine, 2 mmol/l theophylline and 40 g/l BSA. GLP-1-(1-37), GLP-1-(1-36)-peptide amide or glucagon were added at different concentrations in a total volume of 500 µl. At the end of the incubation period, the cell suspensions were treated twice with 0.5 ml of 100 g/l trichloroacetic acid, and the combined supernatants were washed with ether saturated with water.

Cyclic AMP was assayed by radioimmunoassay (Rianen cAMP [¹²⁵I] RIA Kit, New England Nuclear Chemicals, Dreieich, FRG).

Results

Under the conditions of incubation and separation, non-specific binding of ¹²⁵I-glucagon was less than 2% of the total added. While unlabelled glucagon caused 50% displacement of ¹²⁵I-glucagon (ED₅₀) from its specific receptors at a concentration of 3×10^{-9} mol/l, neither GLP-1-(1-37) nor GLP-1-(1-36)-peptide amide at concentrations up to 10^{-6} mol/l displaced ¹²⁵I-glucagon (Fig. 1, upper panel).

When the liver membranes were incubated in the presence of ¹²⁵I-GLP-1-(1-37) at a concentration of 0.85×10^{-9} mol/l, there was no displaceable binding of peptide to the membranes. Non-specific binding (in the presence of 2.4×10^{-7} mol/l unlabelled peptide) was 3.2% of the total tracer added.

Figure 1, lower panel, shows the effect of glucagon, GLP-1-(1-37) and GLP-1-(1-36)-peptide amide on the

Fig. 1 Upper panel: displacement of ¹²⁵I-glucagon from liver membranes by unlabelled glucagon (●), GLP-1-(1-37) (■) or GLP-1-(1-36)-peptide amide (▲). B/B₀ is [tracer bound]/[tracer bound in absence of added peptide]. Lower panel: production of cyclic AMP by isolated rat hepatocytes in the presence of glucagon (●), GLP-1-(1-37) (■) or GLP-1-(1-36)-peptide amide (▲), expressed as percentage of maximum increment produced by glucagon. Effect of adding GLP-1-(1-37) (□) or GLP-1-(1-36)-peptide amide (Δ) at 10⁻⁶ mol/l in the presence of glucagon 3 × 10⁻⁹ mol/l

production of cyclic AMP by isolated liver cells. While glucagon stimulated the production of cyclic AMP over the same concentration range and with the same ED₅₀ (3 × 10⁻⁹ mol/l) as for its interaction with specific glucagon receptors, neither GLP-1-(1-37) nor GLP-1-(1-36)-peptide amide stimulated cyclic AMP production. When the hepatocytes were incubated with 3 × 10⁻⁹ mol/l glucagon together with either GLP-1-(1-37) or GLP-1-(1-36)-peptide amide at 10⁻⁷ and 10⁻⁶ mol/l, the addition of the GLP-1 did not alter glucagon-stimulated production of cyclic AMP.

Discussion

The fact that GLP-1 does not interact with glucagon receptors in rat liver membranes is in accordance with a previous report [7], and the absence of specific, high-affinity GLP-1 receptors in these membranes suggests that the liver is not a target organ for GLP-1. This is confirmed by the finding that GLP-1 does not stimulate cyclic AMP production by isolated hepatocytes, and does not affect glucagon-stimulated cyclic AMP production. It is, of course, possible that the incubation conditions required to demonstrate specific hepatic receptors for GLP-1 differ somewhat from those producing optimal

specific binding of ¹²⁵I-glucagon label. However, the results obtained are consistent with its lack of effect on plasma glucose concentrations at high doses in vivo [6]. Furthermore, the pancreas only produces small amounts of free GLP-1 [5]. All these data suggest that, despite its structural similarity with glucagon, the highly conserved GLP-1 must have a different biological role which would not include a direct action on hepatic carbohydrate metabolism. An intra-pancreatic or enteroinsular role for GLP-1 has been postulated on the basis of its glucose dependent insulinotropic action on isolated rat islets [11]. However, receptors for GLP-1 have not yet been identified in endocrine pancreatic tissue. The evolutionary constraint that has conserved the structure of GLP-1 as completely as that of glucagon must arise from a physiological action that remains to be discovered.

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Professor S. R. Bloom
Second Floor, Francis Fraser Laboratory
Department of Medicine
Royal Postgraduate Medical School
Du Cane Road
London W12 0HS
UK