Diabetologia (1992) 35: 701–711

Diabetologia

Review

Glucagon-like peptide-1, a new hormone of the entero-insular axis

C. Ørskov

Department of Clinical Chemistry, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark

Summary. The post-translational processing of proglucagon in the small intestine gives rise to glucagon-like peptide-1 (PG 78-107 amide) which has profound effects on the endocrine pancreas, and in many species also on the stomach. Glucagon-like peptide-1 (PG 78-107 amide) is secreted in man in response to physiological stimuli e.g. a mixed meal. Glucagon-like peptide-1, in concentrations corresponding to those observed in response to meals, strongly stimulates insulin secretion, in all mammals studied, even more potently than the gastric inhibitory peptide. Thus, glucagon-like peptide-1 fulfills the classic criteria for being a hormone and is likely to be a new incretin. The glucagon inhibitory effect of glucagon-like peptide-1 (PG 78-107 amide) probably further potentiates the effect of glucagon-like peptide-1 on glucose metabolism and distinguished this peptide from other intestinal peptides which have been proposed as incretins. Glucagon-like peptide-1 also inhibits gastric acid secretion and gastric emptying in man. The latter delays nutrient entry to the intestine and thereby diminishes meal-induced glucose excursions. Elevated plasma concentrations of immunoreactive glucagon-like peptide-1 have been reported in Type 2 (non-insulin-dependent) diabetic patients, however, the consequences of the elevation are not yet known. However, elevated levels of glucagon-like peptide-1 in patients with increased gastric emptying rate (post-gastrectomy syndromes) may be responsible for the exaggerated insulin secretion seen in these patients.

Key words: Glucagon-like peptide-1, proglucagon, incretin, insulin, glucagon, somatostatin.

All peptide hormones have been shown to derive from much larger percursor molecules, the "so called" prohormones. Thanks to gene technology the amino acid sequences of many of these prohormones are now known. Apart from the actual hormone sequences, many prohormones have been found to contain additional, up to now, totally unknown peptide sequences. As exemplified by pro-opiomelanocortin these additional peptides may have new interesting biological effects. One such prohormone is the prohormone glucagon, proglucagon.

The structure of anglerfish proglucagon (PG) was published by Lund et al. in 1982 [1]. Its structure was deduced from the nucleotide sequence of cDNA from anglerfish islets encoding the precursor of glucagon. Apart from the glucagon sequence the prohormone was shown to contain an N-terminal amino acid sequence of 52 amino acids and a C-terminal 34 amino acid glucagon-like sequence [1]. The structure of the first mammalian PG deduced from the nucleotide sequence of glucagon-encoding mRNA from hamster pancreatic islets was published by Bell et al. in 1983 [2]. Apart from glucagon, proglucagon was shown to contain a 91-amino acid C-terminal sequence containing two glucacon-like sequences, flanked by pairs of basic amino acids, known as potential cleavage sites. The peptides were named glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-2 (GLP-2) [2]. The discovery of the two new glucagon-like sequences initiated a surge of studies. There were numerous questions to answer: were the glucagon-like peptides produced in the tissues, would these peptides be released by the producing cells and by which stimuli, and last, but not least, could any of these peptides have a biological function, and would this function then be glucagon-like? Many workers have contributed to the attempts to answer these questions from 1983 till today. Not all questions are yet answered but the progress made so far has important implications. It is now generally accepted that the naturally occurring GLP-1 (a peptide corresponding to PG 78-107 amide), secreted from the distal ileum, has unique effects on the endocrine pancreas and thereby potentially affects glucose metabolism. This GLP-1 molecule has been postulated to be a novel "incretin". Incretin is a term coined by Zunz and La Barre in 1929 for a humoral factor from the intestinal tract which releases insulin or potentiates the glucose-induced

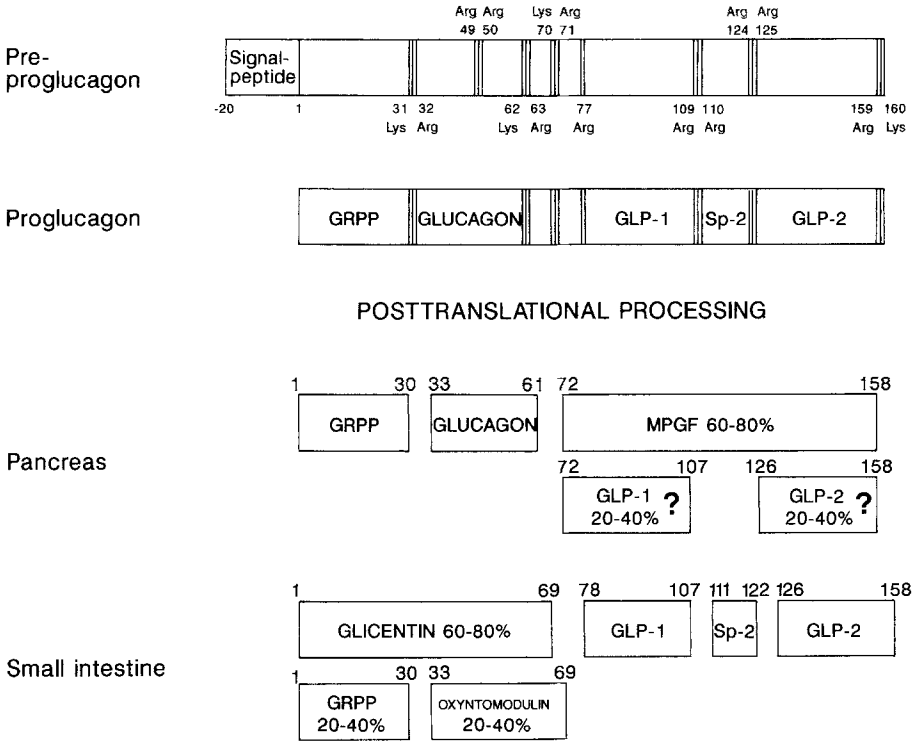

Fig. 1. *Proglucagon processing in mammalian pancreas and small intestinal mucosa.* The structure of the glucagon precursor is indicated diagrammatically at the top. Sequences of dibasic amino acids, potential post-translational cleavage sites, are indicated. The peptide products are shown in boxes, marked according to their position in the prohormone sequence in the upper corners of the boxes together with the name. The relative amount of a given peptide sequence occurring in each organ is indicated as per cent of the total amount of proglucagon processed. In the pancreas the main products are glucagon, the N-terminal 30 amino acid peptide, which in the pig has been named glicentin-related pancreatic peptide (GRPP), and the major proglucagon fragment (MPGF). Smaller amounts of not fully characterized glucagon-like peptide-1 and -2 (GLP-1 and GLP-2) immunoreactivities are also formed. In the small intestine the main products are glicentin, GLP-1 (PG 78-107 amide), GLP-2 and a small intervening peptide: spacer peptide-2 (Sp-2)

insulin release [3]. The ability of an oral glucose load to release more insulin than an intravenous glucose infusion, despite a similar increase in the blood glucose level is due to the release of "incretin" [4]. The chemical structure of "incretin" has not yet been determined with certainty, but gastric inhibitory polypeptide, also called glucose-dependent insulin releasing polypeptide or GIP which is produced in the upper small intestine could serve as an incretin. However, it seems probable that several peptides acting together might account for the incretin effect. In fact, in 1981 Lauritzen et al. [5], who studied the incretin effect in patients with small intestinal resections, found that patients with residual ileal segments had greater incretin effects than patients with small jejunal remnants and no ileum, despite identical GIP levels in the two groups, and concluded from these findings that an unknown incretin factor must be present in the distal small intestine [5].

This review analyses the published literature on glucagon-like peptides in order to document these proposals and discusses the evidence that GLP-1 (PG 78-107 amide) is a true hormone.

Literature about glucagon biosynthesis published before the discovery of the full proglucagon structure is generally not covered by this review. The reader is referred to the complete literature review published in 1983 [6].

The structure of preproglucagon

Shortly after the publication of the mammalian proglucagon structure [2] Bell and co-workers described the human preproglucagon gene to be a 9.4 kilobase polynucleotide, presumably composed of six exons (540 base pairs) and five introns [7, 8]. Bell and co-workers [2] proposed that the amino acid sequence of the preprohormone, as deduced both from the hamster cDNA and from the human gene, is composed of a 20 amino acid signal peptide, followed by a 160 amino acid prohormone, proglucagon (PG 1-160). The amino acid sequences of bovine, rat, guinea pig and degu proglucagon were found to be very similar to hamster preproglucagon [9–12]. In the original report, Bell et al. missed the sixth exon of the human glucagon gene, whereby the C-terminal arg (arg 160) could not be predicted to exist [7]. This was pointed out by Heinrich et al. in 1984 [10] and confirmed by White et al. in 1986 [8]. All known mammalian PGs contain, at the PG residues no. 33–61, the sequence of glucagon (Fig. 1). As a consequence they all contain an N-terminal sequence, corresponding to PG 1-30. In contrast to the findings in anglerfish and chicken where one glucagon-like sequence is found C-terminally [1, 13], mammalian PG has a 91 amino acid sequence containing two glucagon-like amino acid sequences. Proteolytic processing of the C-terminal portion of PG at all pairs of basic amino acid residues, potential post-translational cleavage sites, would result in two peptides, each containing a glucagon-like sequence, and a short intervening peptide. Bell and co-workers in 1983 named these peptide sequences GLP-1 (corresponding to PG 72-108), GLP-2 (corresponding to PG 126-158 or 126-160 if the two C-terminal basic amino acids were not removed) and spacer peptide-2 (corresponding to PG 111-122) [7] (Fig. 1). The degree of sequence homology of PG between species varies in the different PG regions: the weakest homology is found in the region containing the NH_2-terminal 30 amino acid fragment (e.g. 84% homology man/hamster). The homology

His - **Ala** - **Glu** - *Gly* - *Thr* - *Phe* - *Thr* - *Ser* - *Asp* - **Val** -

Ser - **Ser** - *Tyr* - *Leu* - **Glu** - **Gly** - **Gln** - **Ala** - *Ala* - **Lys** -

Glu - *Phe* - **Ile** - **Ala** - *Trp* - *Leu* - **Val** - **Lys** - **Gly** - **Arg** - **NH_2**

Mammalian glucagon-like peptide-1 (PG 78-107 amide)

Fig. 2. *The amino acid structure of mammalian glucagon-like peptide-1 (PG 78-107 amide).* Amino acids shown in italic letters occur at the same position in the sequence of glucagon

in the region containing the GLP-2 sequence is higher (91% homology man/hamster), but the highest homology is found in the two regions containing the glucagon sequence and the GLP-1 sequence (both 100% homology man/hamster) [2, 7]. Actually, the amino acid sequences of GLP-1 are identical in all mammals studied so far (Fig. 2). This high degree of conservation would be consistent with similarly conserved biological functions.

Interestingly, anglerfish have been shown by Lund et al. [14] to have two glucagon genes encoding two different proglucagon molecules. Both of the anglerfish PG molecules contain one GLP-1 like sequence (both with an N-terminal six amino acid deletion, compared with mammal GLP-1), but neither of the PG molecules contain a GLP-2 sequence. This finding might indicate that GLP-2 is a more recent addition in evolution. However, the occurrence of two glucagon genes in anglerfish could also represent a different mechanism for generating two GLP's such as in mammals [14].

The preproglucagon gene has been shown to be expressed both in the pancreas and the intestine [15]. By cloning and sequencing of cDNAs coding for preproglucagon from human pancreas and colon, combined with primer extension analysis of mRNA from pancreas, ileum and colon, Novak et al. [15] found that the mRNAs for preproglucagon are identical in the pancreas and in the gut. A similar conclusion was reached by Mojsov et al. [16] who studied hybridizing intestinal and pancreatic rat mRNAs by Northern blot analysis. This would be consistent with the predictions by Bell et al. [7] and White et al. [8] that there is only a single gene encoding glucagon in mammals.

However, long before the elucidation of the complete PG structure it had been shown by purification and sequence analysis of pancreatic and intestinal peptides that the peptides produced in the glucagon-cells in the pancreas and in the gut were different. The pancreatic Alpha cells produce glucagon and the 30 amino acid N-terminal peptide (named glicentin-related pancreatic peptide, in the pig), while the L-cells in the small intestine produce glicentin (or "enteroglucagon", corresponding to PG 1-69) and oxyntomodulin (corresponding to PG 33-69) (Fig. 1) [17]. Since the glucagon-encoding mRNAs from pancreas and intestine are identical, the occurrence of different N-terminal PG products in the two tissues can only be explained by tissue-specific, differential post-translational processing of the prohormone.

In some species glucagon-immunoreactive peptides have been identified in the stomach (dog) and in the brain (rat, dog and neonatal human). A cDNA encoding PG transcribed from the nucleotide sequence of human neonatal brainstem mRNA has been shown by Drucker et al. [18] to be identical to PG encoding cDNA from the pancreas and intestine. The sequence of preproglucagon encoding nucleotides in the dog stomach is not known, but it is believed that these products are also derived from the single glucagon gene.

Nomenclature

As discussed later, there is more than one naturally occurring GLP-1 immunoreactive molecule, and many synthetic GLP-1 analogues are now available. Their nomenclature is quite confusing, since the different workers use different names for the same molecular forms. To meet these inconsistencies it was decided in this review, to designate the peptides according to their position in the prohormone sequence (PG 1-160) together with the name, (eg. GLP-1 (PG 78-107 amide)).

GLP-1 (PG 78-107 amide) has the following synonyms: GLP-1 (7-36 amide), intestinal GLP-1, and truncated GLP-1 (TGLP-1). GLP-1 (PG 78-108) is synonymous with GLP-1 (7-37) and "insulinotropin". GLP-1 (PG 72-107 amide) is synonymous with GLP-1 (1-36 amide) or "full-length GLP-1". The presence or absence of C-terminal amidation of these moieties depends on whether it is believed that the PG sequence no. 108–110, gly-arg-arg, a potential amidation site, is substrate for an amidation reaction or not.

The large pancreatic GLP-1-containing molecule will, as first suggested by Patzelt and Schug [19], be designated the major proglucagon fragment (MPGF), as its exact sequence is not yet known.

Molecular characterization of naturally occurring peptides containing the GLP-1 sequence (PG 78-107) (GLP-1 molecules)

Pancreatic GLP-1 molecules

In 1979 Patzelt et al. [20] identified a 10,000 M_r proglucagon fragment from rat pancreatic islets, which he named the major proglucagon fragment (MPGF). By comparing the amino acid composition of MPGF and the known structure of rat PG, Patzelt et al. [21] later deduced that rat MPGF probably corresponds to PG 72-158. Also GLP-1 molecules in pancreata from pig, dog and man have been studied by chromatography [16, 22–30]. The available evidence suggests that the main GLP-1 moiety produced in the pancreas is indeed a large peptide, which contains both the GLP-1 and the GLP-2 sequences. The reported presence of smaller pancreatic GLP-1 molecules may result from enzymatic degradation of larger peptides prior to gel filtration or from inexpedient extraction procedures or specificities of the antisera used for the measurements [16, 22–33].

Enteric GLP-1 molecules

Because the largest number of enteroglucagon producing cells had previously been found in the ileum and the colon [34, 35], these tissues were examined for GLP-1 molecules in rat [16, 23], pig [24], dog [25] and in man [26, 27, 29]. All workers found that the majority of the GLP-1 immunoreactivity eluted as a small molecule coeluting with either GLP-1 (PG 72-107 or -8) or GLP-1 (PG 78-107 or -8). By sequence analysis of purified peptide from human and porcine intestine, the peptide has been shown to correspond to PG 78-107 amide [36, 37]. Likewise, mass spectroscopy of purified rat GLP-1 was compatible with the theoretical mass of PG 78-107 amide [38]. In conclusion, it is apparent that both the N-terminal and the C-terminal parts of PG are processed differentially in the pancreas and the small intestine.

Gastric GLP-1 molecules

By chromatographic analysis of extracts of canine gastric mucosa, known to produce glucagon [39, 40] almost all of the GLP-1 immunoreactivity eluted at a position corresponding to the MPGF.

Cerebral GLP-1 molecules

GLP-1 immunoreactivity has been found in whole brain extracts, and brain stem and hypothalamus extracts from rat and dog [18, 25, 41–43]. By chromatographical analysis the immunoreactivity eluted corresponding to either GLP-1 (PG 72-107 amide) or (PG 78-107 amide). The significance of the presence of GLP-1 in the brain is not known.

Cellular localization of GLP-1 molecules

Pancreas

In mammals GLP-1 immunostaining is found in the islets of Langerhans, co-localized with glucagon and GLP-2 immunostaining [16, 24, 27, 29, 44, 45]. By electron microscopy the different products of PG have been shown to be packaged in separate compartments of the secretory granules in the pancreatic Alpha-cells: GLP-1, GLP-2 and glucagon immunostaining in the electron dense core, and immunostaining for the 30 amino acid N-terminal product of PG in the less electron dense halo [16, 46, 47]. A totally different peptide, pancreastatin, (an amidated fragment of chromogranin A) has also been found in the Alpha cells, packaged in the less electron dense halo of the secretory granules [48]. The significance of the differential packaging of the PG products in the secretory granules and the coexistence with pancreastatin is unknown.

Gastrointestinal tract

In the gastrointestinal tract GLP-1 and GLP-2 immunostaining co-localized with glicentin/enteroglucagon immunostaining has been shown in open-type endocrine cells in ileal mucosa in all mammals studied so far [16, 24, 27, 44], including human colonic [46] and rectal mucosa [45]. Peptide YY (PYY) immunostaining has been shown to be co-localized with glicentin immunostaining (and therefore probably also with GLP-1 immunostaining) in all parts of the intestine [49–51]. The significance of the coexistence of PYY and the PG products is unknown.

Stomach

In tissue sections of dog gastric mucosa, known to harbour glucagon-producing cells [40], GLP-1 immunostaining has been shown to be co-localized with glucagon immunostaining [44].

Brain

The finding of cells in the nucleus tractus solitarius in rat brainstem which hybridize with a GLP-1 oligonucleotide probe suggests that there is a de novo synthesis of PG in these cells [52]. Likewise, GLP-1 immunostaining co-localized with glucagon and glicentin immunostaining has been shown in cells of the nucleus of the solitary tract in rat brainstem [53], in the primate, *Tupaia Berlangeri* [45] and in man. The reported occurrence of GLP-1 immunostaining cells in rat hypothalamus is more doubtful [18, 53, 54].

Secretion of GLP-1 molecules

Secretion of GLP-1 molecules and stimuli for the secretion of GLP-1 molecules have been studied in isolated perfused organs from pig, rat and dog, and in vivo in man.

Pancreatic secretion of GLP-1 molecules

The amino acid, arginine, has been shown to stimulate the secretion of GLP-1 immunoreactivity from rat islets [19, 21], isolated perfused rat pancreas [22, 55] and isolated perfused pig pancreas [24]. The secreted GLP-1 immunoreactivity was characterized chromatographically and found to correspond to a large molecule, probably the MPGF, by some workers [21, 24], though Shima et al. [55] reported that most of the GLP-1 immunoreactivity coeluted with GLP-1 (PG 72-107 amide).

Intestinal secretion of GLP-1 molecules

The secretion of GLP-1 immunoreactivity has been shown to be stimulated by intraluminal glucose in isolated perfused pig ileum [24], and isolated perfused dog ileum [56, 57] and by the neuropeptide gastrin releasing peptide in isolated perfused pig ileum [24]. By chromatographic analysis the secreted GLP-1 immunoreactivity was reported by all workers to correspond to a relatively small molecule coeluting either with GLP-1 (PG 72-107 amide) [24] or GLP-1 (PG 78-107 amide) [56].

Shima et al. [58] investigated the relationship between the molecular structures of sugars and their ability to

stimulate the release of immunoreactive GLP-1 from ileal loops in anaesthetized dogs. They reported that D-glucose, D-galactose, D-glucuronic acid, 3-O-methyl-D-glucose, maltose, sucrose and maltitol stimulated the release of GLP-1 immunoreactivity while D-fructose, D-fucose, D-xylose, D-mannose and, surprisingly, lactose did not. The common chemical features of those sugars that stimulated GLP-1 secretion were reported to be an electron density near C (6), an equatorial hydroxyl group at C (2), and an axial hydroxyl group at C (1). In conclusion, GLP-1 immunoreactive material is released both from the pancreas and from the small intestine in response to physiological stimuli also known to cause the release of glucagon/glicentin. From the pancreas a large GLP-1 immunoreactive molecule probably corresponding to the MPGF is secreted, whereas the small intestine mainly secretes a smaller molecule, corresponding to GLP-1 (PG 78-107 amide) or GLP-1 (PG 72-107 amide). The present knowledge, that the extractable intestinal GLP-1 in man, rat and pig is GLP-1 (PG 78-107 amide), makes it very likely that the GLP-1 immunoreactive moiety secreted from the intestine is also GLP-1 (PG 78-107 amide).

Circulating GLP-1 molecules in normal man

The secretion of GLP-1 molecules in man has been studied through measurements of peripheral venous plasma. Most workers have found that accurate measurements of GLP-1 in plasma require previous extraction of plasma to remove unspecific protein interference in the radioimmunoassay [30, 59]. Unspecific interference by plasma proteins, which is also a problem in many other radioimmunoassays, has been studied in detail by several groups (see [60] and [61] for glucagon and gastrin radioimmunoassays).

Some disagreement prevails as to which GLP-1 molecules predominate in fasting plasma. Some workers have found that in the fasting state the predominating GLP-1 molecule in plasma behaves chromatographically like MPGF [27, 62, 63], while other workers reported that the predominating molecule corresponds to GLP-1 (PG 72-107 amide) [28, 30]. The disagreement could be due to the different chromatographic techniques employed. Thus, some workers have reported that for example Seppak used in many studies retains the MPGF [28, 62].

Both Kreymann et al. [30] und Ørskov et al. [27, 59, 62] found that the concentration of GLP-1 immunoreactivity in plasma rose in response to a mixed meal. By chromatographic analysis of postprandial plasma Ørskov et al. [27] found that the majority of the GLP-1 immunoreactivity eluted at the position of synthetic GLP-1 (PG 72-107 amide) or GLP-1 (PG 78-107 amide), which could not be separated by this gel filtration procedure.

Intravenous arginine, an established stimulus for the pancreatic glucagon-secreting cells, has been shown by several workers to stimulate GLP-1 secretion in man [30, 62]. By chromatographic analysis of plasma this GLP-1 immunoreactivity was shown to correspond to a large peptide, probably the MPGF.

Oral glucose, a known stimulus for the glicentin/GLP-1 producing cells in the intestinal mucosa, was also shown to stimulate GLP-1 immunoreactivity in peripheral plasma [32, 62]. By chromatographic analysis it was shown that this GLP-1 immunoreactivity in plasma corresponded to GLP-1 (PG 78-107 amide).

In conclusion, the predominating GLP-1 in plasma after a mixed meal and after oral glucose coelutes with GLP-1 (PG 78-107 amide), reflecting a stimulation of the gut. After intravenous arginine the main GLP-1 molecule in plasma is the MPGF, reflecting a stimulation of the endocrine pancreas.

Circulating GLP-1 molecules in disease

Circulating GLP-1 molecules in disease have so far only been studied in a few conditions, namely in Type 2 (non-insulin-dependent) diabetic patients, in gastrectomized patients, in uraemic patients and in patients with the glucagonoma syndrome.

GLP-1 molecules in Type 2 diabetes

GLP-1 immunoreactivity in plasma in the fasting state, following oral glucose and intravenous arginine has been shown to be significantly higher in Type 2 diabetic patients than in matched control subjects [62, 63]. By chromatographic analysis of plasma from the Type 2 diabetic patients it was shown that the predominating GLP-1 molecule both in the fasting state and after intravenous arginine was a large molecule, corresponding to the MPGF, whereas after oral glucose the predominating GLP-1 molecule in plasma coeluted with synthetic GLP-1 (PG 78-107 amide) [62, 63].

GLP-1 molecules in gastrectomized subjects

Miholic et al. [30] studied the effects of a meal and Kreymann et al. [64] studied the effects of oral glucose on GLP-1 secretion in gastrectomized subjects after meal/glucose stimulation. In both studies a significantly higher GLP-1 concentration was measured in the gastrectomized subjects than in the control subjects. By chromatographic analysis both groups found that this GLP-1 immunoreactivity corresponded to GLP-1 (PG 78-107 amide). It was speculated by Miholic et al. [64] that the elevated GLP-1 concentrations might account for the exaggerated insulin secretion seen in these patients in response to a meal, which sometimes results in reactive hypoglycaemia.

GLP-1 molecules in uraemic subjects

GLP-1 immunoreactivity in fasting plasma from uraemic patients has been shown to be significantly higher than in control subjects [65]. By chromatographic analysis the GLP-1 immunoreactivity eluted in two equally large peaks corresponding to the MPGF and a moiety co-elut-

Table 1. Elimination of exogenous glucagon-like peptide (GLP) (PG 72-107 amide) and GLP 1 (PG 78-107 amide)

Species	Peptide	T 1/2 (min)	MCR ($ml \cdot kg^{-1} \cdot min^{-1}$)	Authors	Comments
Rat	GLP	8.2 ± 0.4	Not determined	Ruiz-Grande et al. [66]	Single bolus injections
Rat	GLP	48 ± 14	27	Oshima et al. [67]	The volume of distribution calculated for these values > 1000 ml/kg
Man	GLP	17 ± 2	2.2 ± 0.3	Scholdager et al. [68]	Continuous infusion
Rat	GLP 1	4.5 ± 0.2	Not determined	Ruiz-Grande et al. [69]	Single bolus injection
Man	GLP 1	4	12	Kreymann et al. [30]	Infused continuously
Man	GLP 1	11 ± 2	13 ± 3	Scholdager [68]	Infused continuously

T 1/2, plasma half-life; MCR, metabolic clearance rate

ing with GLP-1 (PG 72-107 amide). This finding suggests that the kidneys play a role in the removal of these PG products from plasma.

GLP-1 molecules in patients with glucagon-producing tumours

Uttenthal et al. [29] and George et al. [26] studied GLP-1 molecules in tumour tissue and plasma from patients with glucagon producing tumours. The concentration of GLP-1 immunoreactivity was elevated in plasma samples and in all extracts of tumour tissue. By chromatographic analysis of both plasma and extracted tissue the size of GLP-1 molecules varied from patient to patient.

Metabolism of GLP-1 molecules

The plasma half-lives and metabolic clearance rates of both exogenous GLP-1 (PG 72-107 amide) and GLP-1 (PG 78-107 amide) have been studied by several workers [30, 66–69]. The results of the studies in rat and man are shown in Table 1. In comparison the metabolic clearance rate of glucacon is 9–12 $ml \cdot kg^{-1} \cdot min^{-1}$ and the half-life is 5–6 min. Thus, the half-life of GLP-1 is only slightly longer than that of glucagon.

Biological effects of GLP-1 molecules

The biological effects of GLP-1 molecules have been studied in isolated organs from rat and pig and in vivo in man.

Biological effect of major proglucagon fragment (MPGF)

No studies on the effect of MPGF have been published. The exact structure is not known, and hence a synthetic replica of the peptide is not available for biological studies.

Biological effect of GLP-1 (PG 72-107 amide)

Synthetic GLP-1 (PG 72-107 amide) in very high concentrations (above 1 nmol/l) was found to stimulate insulin secretion from isolated rat pancreatic islets [70] and from isolated perfused rat pancreas [71], whereas synthetic GLP-1 (PG 72-107 amide) in isolated perfused pig pancreas, in concentrations up to 10^{-9} mol/l, neither affected the exocrine secretion, nor the secretion of insulin, glucagon, somatostatin or GLP-2 from the endocrine pancreas [24].

GLP-1 (PG 72-107 amide) in very high concentrations (10^{-8} to 10^{-6} mol/l) was reported to stimulate glycerol release from isolated rat adipocytes, but much less potently than glucagon [72].

Intravenous infusion of synthetic GLP-1 (PG 72-107 amide) in doses resulting in supraphysiological peripheral concentrations of GLP-1 (500 pmol/l) in healthy subjects slightly inhibited pentagastrin-induced acid secretion [68].

A few studies have been published describing binding sites for GLP-1 in the rat brain. Shimizu et al. [42] reported specific binding of GLP-1 (PG 72-107 amide) to homogenates of rat pituitary glands and thalamus/hypothalamus and Hoosein and Gurd [73] found that GLP-1 (PG 72-107 amide) at 10^{-9} mol/l increased the cAMP formation two-fold in membrane preparations of rat hypothalamus and pituitary.

Both Ghiglione et al. [74] and Shimizu et al. [75] reported that GLP-1 (PG 72-107 amide) did not, like glucagon, bind to rat hepatocyte membranes, and did not stimulate the production of cAMP in isolated rat hepatocytes. Likewise, Ørskov et al. [unpublished results] found that GLP-1 (PG 72-107 amide) did not have specific binding sites on porcine hepatocyte membranes.

In conclusion, GLP-1 (PG 72-107 amide) in physiological concentrations has no known biological effect. Supraphysiological concentrations of GLP-1 may affect insulin secretion, lipid metabolism and acid secretion, but all of these effects may be due to degradation of the peptide to GLP-1 (PG 78-107 amide). The significance of the putative receptors on brain membranes is not known.

Biological effects of GLP-1 (PG 78-107 amide/108)

a) Effects on the endocrine pancreas. GLP-1 (PG 78-107 amide) was first shown in 1987 [36] to stimulate insulin secretion in the isolated perfused porcine pancreas. Later GLP-1 was shown to have a similar effect in isolated perfused dog pancreas [76] and rat pancreas [71, 76–79],

mouse pancreas [80] and in concious sheep [81]. The non-amidated peptide GLP-1 (PG 78-108) has also been shown to stimulate insulin secretion in perfused rat pancreas [82–85] and in concious rats [84]. The insulin stimulatory effect of both GLP-1 (PG 78-107 amide) and GLP-1 (PG 78-108) has been shown to be glucose-dependent [71, 79, 82]. Both Shima et al. [71] and Suzuki et al. [86] compared the effects of GLP-1 (PG 78-107 amide) and gastric inhibitory peptide, GIP, an incretin candidate from the upper small intestine, on insulin secretion in the isolated perfused rat pancreas. While Shima et al. [71] reported that GLP-1 was much more potent than GIP, Suzuki et al. [86] claimed that the two peptides (both infused at 10^{-9} mol/l) were equipotent.

The insulin stimulatory effect of GLP-1 (PG 78-107 amide) has also been studied in man. Kreymann et al. [30] found by intravenous infusion of GLP-1 together with glucose that insulin secretion was stimulated greatly and Nathan [87] found that infusion of GLP-1 increased insulin secretion two-fold in healthy volunteers. The effects of GLP-1 (PG 78-107 amide) and GIP on insulin secretion in healthy volunteers have been studied by several workers [30, 88–90]. In all studies both GIP and GLP-1 stimulated insulin secretion, but GLP-1 was more potent than GIP, and GLP-1, in contrast to GIP, had insulinotropic actions at euglycaemia [30, 88]. Furthermore, Nauck et al. [89] showed that the insulin response in normal subjects after an infusion of GLP-1 and GIP in physiological concentrations together with intravenous glucose, corresponded to the insulin response to a matched oral glucose load. Thus, by addition of these peptides to the intravenous glucose the incretin effect was mimicked. Interestingly, the incretin effect, as well as the secretion of GIP and GLP-1 after an oral glucose load, have been shown to be preserved in diabetic patients after pancreas transplantation, in spite of the denervation of the pancreas and the systemic insulin delivery [91]. Gutniak et al. [92] studied the effect of GLP-1 on isoglycaemic meal-related insulin requirement in Type 2 and Type 1 (insulin-dependent) diabetic patients. They found that the insulin requirements after GLP-1 infusion in both the Type 2 and the Type 1 diabetic patients were greatly reduced and speculated that GLP-1 might be a new treatment for diabetes [92].

Thus, GLP-1 is, also in man, the most potent endogenous insulin-stimulating peptide isolated to date, considerably more potent than GIP. In physiological concentrations GLP-1 is more potent at hyperglycaemia and euglycaemia than at hypoglycaemia. The glucose-dependency of the effect of GLP-1 (PG 78-107 amide) is similar to that of GIP.

GLP-1 (PG 78-107 amide) was also found to stimulate somatostatin secretion in a perfused pig pancreas [93] and dog pancreas [76], and in a rat pancreatic islet cell culture [94].

Finally, GLP-1 (PG 87-107 amide) in physiological concentrations has been shown to inhibit glucagon secretion from isolated perfused pancreas in the pig [93], in the dog [76] and in the rat [76, 77, 79]. The inhibitory effect was reported to be more marked at low than at high glucose levels [76, 79, 93]. Interestingly, GLP-1 (PG 78-107 amide) did not affect glucagon secretion in a rat pancreatic cell culture [94]. This finding might indicate that the effect of GLP-1 (78-107 amide) on insulin and somatostatin secretion is direct, while the effect on glucagon secretion may be indirect (a paracrine effect mediated via somatostatin?) and therefore may require intact pancreatic islets.

The non-amidated GLP-1 (PG 78-108) has been shown by Weir et al. [83] not to inhibit glucagon secretion.

In man, Kreymann et al. [30] found that GLP-1 (PG 78-107 amide) infused intravenously into healthy volunteers together with glucose significantly inhibited glucagon secretion.

b) Effects on the stomach. The effect of GLP-1 (PG 78-107 amide) on the stomach has been studied because of the known inhibitory effect of glucagon on gastric acid secretion [95]. While GLP-1 was shown to neither affect somatostatin secretion nor gastrin secretion in the pig stomach [93], the peptide was found to stimulate somatostatin release and inhibit gastrin release in a perfused rat stomach [96, 97]. These latter effects were not abolished by tetrodotoxin, indicating that the effects were not neurally mediated [96]. Schmidtler et al. [98] have reported a direct stimulatory effect of GLP-1 on dispersed rat parietal cells.

Reports on the occurrence of GLP-1 receptors in the stomach are contradictory: strong specific binding has been reported [99, 100] as well as weak binding [101] and no specific binding [102].

In man, GLP-1 in physiological concentrations has been shown to inhibit gastric acid secretion [68, 103, 104] and to decrease gastric emptying [104]. It is not known if the inhibitory effect of GLP-1 on acid secretion in man is direct or indirect. The inhibitory effect of glucagon is presumably indirect [105].

c) Possible effects on other tissues. GLP-1 receptors or effects have been looked for in several tissues. Oben et al. [106] recently reported that GLP-1 stimulates fatty acid synthesis in explants of rat adipose tissue. Kanse et al. [101] found high levels of GLP-1 binding to homogenates of rat brain stem and lung, whereas low binding to homogenates of pancreas, liver, adipose tissue, stomach mucosa and spleen was found. Specific binding of GLP-1 to a 66,000 M_r single ligand binding protein isolated from cell membranes of rat lung was reported by Richter et al. [107, 108]. The size of this putative GLP-1 receptor was comparable with the reported size of the GLP-1 receptor on rat insulinoma cells.

Seifert et al. [111] reported specific binding of GLP-1 to homogenates of the superior colliculus, the pretectal area, the nucleus of the solitary tract and the hypothalamus in rats. The significance of the reported GLP-1 binding to rat lung, brain and adipose tissue is not known.

Biological effect of GLP-1 analogues (Table 2)

Suzuki et al. [77] compared the effects of the GLP-1 analogues, PG 72-107 amide, PG 78-107 amide, PG 77-107 amide, and PG 79-107 amide, on an isolated perfused rat

Table 2. Known biological effects of glucagon-like peptide-1 (GLP-1) molecules in physiological concentrations

GLP-1 (PG 78-107 amide/108)
Stimulates insulin secretion (man rat, pig)
Stimulates somatostatin secretion from pancreas (pig, dog, rat)
Inhibits glucagon secretion (man, pig, dog, rat)
Stimulates somatostatin secretion from (rat) stomach
Inhibits gastric acid secretion (man)
GLP-1 (PG 72-107 amide/108)
No known effects
Major proglucagon fragment (PG 72-158?)
No known effects

The species in which the investigations were carried out are indicated in parentheses

pancreas. GLP-1 (PG 78-107 amide) (and very high concentrations of GLP-1 (PG 72-107 amide)) stimulated insulin secretion, while the analogues PG 77-107 amide and PG 79-107 amide did not. Only GLP-1 (PG 78-107 amide) inhibited glucagon secretion in the perfused pancreas [77]. In a study by Gefel et al. [84] the insulin stimulatory effect of GLP-1 (PG 78-107 amide) was found to be similar to that of GLP-1 analogue PG 78-108 in the isolated rat pancreas, while the GLP-1 analogue PG 78-105 was less potent, and PG 78-104 and PG 79-108 did not affect insulin secretion at all. Kawai et al. [112] found that GLP-1 (PG 78-107 amide) stimulates insulin secretion more potently in concious dogs than PG 78-108 and PG 78-106. Gallwitz et al. [113] compared the ability of GLP-1 (PG 78-107 amide) and two fragments corresponding to PG 78-97 and PG 93-107 amide to stimulate cAMP in rat insulinoma cells and found that only GLP-1 (PG 78-107 amide) was able to stimulate cAMP. Yanaihara et al. [97] compared the somatostatin releasing effects of the GLP-1 analogues: GLP-1 (PG 72-108), (PG 78-108), (PG 78-107), (PG 78-106) and (PG 78-104) in an isolated perfused rat stomach. At 10^{-8} mol/l the GLP-1 analogues, PG 78-108, PG 78-107, PG 78-106 and PG 78-104, all stimulated somatostatin secretion significantly, while GLP-1 (PG 72-108) did not stimulate somatostatin.

The results of these studies indicate that an intact N-terminus of GLP-1 (PG 78-107 amide) is very important for the effects on insulin and somatostatin secretion, while an intact C-terminus seems to be important for the effect on insulin secretion.

GLP-1 molecules and GLP-1 receptors in transformed cells

Almost all studies of effects of GLP-1 molecules on transformed cells have been performed in different strains of a rat insulinoma cell line (RIN) originally derived from a radiation-induced insulin-producing rat tumour [114, 115].

Philippe et al. [116] studied PG processing in a cloned rat insulinoma cell line, RIN 56A. By Northern blot analysis this cell line was found to express high levels of glucagon mRNA, and by chromatographical analysis to produce small amounts of a GLP-1 immunoreactive moiety coeluting with synthetic GLP-1 (PG 72-108). Larger molecular weight moieties were not identified. Specific receptors for GLP-1 (PG 78-107 amide) [109, 117] and GLP-1 (PG 78-108) [118, 119] on RIN cells, not displacable by glucagon, GLP-2, GIP or any other member of the glucagon/secretin family have been described by several groups [109, 116–120]. Binding of GLP-1 (PG 78-107 amide) and GLP-1 (PG 78-108) has been shown to be associated with increase in cyclic AMP concentration in the cell medium (in RIN m5F, RIN 1046-38 and RIN 1027-B2) [109, 119] and it was shown that guanine nucleotides significantly decreased receptor affinity to GLP-1 [118]. Furthermore, GLP-1 binding to RIN cells was shown to affect neither the membrane potential nor to change the intracellular levels of calcium [118]. It was concluded that the effect of GLP-1 (PG 78-107 amide) on RIN cells seems to be mediated solely by G-protein binding receptors associated with adenylate cyclase [118]. By covalent cross-linking of ^{125}I-labelled GLP-1 to RIN 5mF cell membranes Göke et al. demonstrated a single, ligand-binding protein of a molecular weight of 63,000, thought to be a GLP-1 receptor [121]. In a hamster islet cell line, InR1-G9, Drucker et al. [122] found by gel filtration of extracted cells, that all GLP-1 immunoreactivity eluted at the position of synthetic GLP-1 (PG 72-108) and that phorbol myristate acetate stimulated the release of this GLP-1 immunoreactive moiety, suggesting that protein kinase C activating mechanisms were involved.

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Dr. C. Ørskov
Department of Clinical Chemistry KK 3011
Rigshospitalet
Blegdamsvej 9
DK-2100 Copenhagen
Denmark