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Reviews

Proglucagon-derived peptides: nomenclature, biosynthetic relationships and physiological roles

J. M. Conlon

Clinical Research Group for Gastrointestinal Endocrinology of the Max-Planck-Gesellschaft, University of Göttingen, FRG

The decade of the 1980s has seen enormous advances in the field of molecular endocrinology brought about in large measure by application of the methods of recombinant DNA technology. The increase in our knowledge and understanding of the glucagon-related peptides provides an excellent illustration of this point. Cloning and sequence analysis of cDNAs and DNA fragments from genomic libraries has led to the elucidation of the primary structure of the biosynthetic precursor of glucagon (pre-proglucagon) from a variety of species including man [1–6]. With this information, it has been possible to interpret the complex structural relationships between the multiple forms of the glucagon-like peptides in tissues and in the circulation. Probably the most exciting development in the field is the realisation that the pre-proglucagon gene in mammals encodes, in addition to glucagon, two additional peptides with structural similarity to glucagon, termed glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-2 (GLP-2). This review attempts to summarise recent advances with a view to clarifying previous ambiguities regarding the nomenclature and biosynthetic relationships between the peptides and speculates as to the physiological role of the peptides.

Nomenclature of proglucagon-derived peptides

A previous attempt [7] to develop a workable nomenclature for the classification of glucagon-related peptides was based upon a system that characterised a peptide by its approximate molecular weight, generally determined by gel permeation chromatography, and by its reactivity towards antisera to glucagon of defined regional specificity. Glucagon, for example, was described as IRG^{3500}. This nomenclature is subject to severe limitations as the specificities of available antisera are seldom totally clear and apparent molecular weights may be wildly inaccurate. The primary structures of the biosynthetic precursors of most of neurohormonal peptides are now known from the nucleotide sequences of cDNAs or gene fragments. It becomes possible, therefore, to use an unambiguous nomenclature that is based either on the amino acid sequence of the prohormone or, when the site of cleavage of the signal peptide is not known, on the sequence of the pre-prohormone. Even when experimental evidence is lacking, the site of signal peptide cleavage may be predicted with a high degree of probability [8] so that a nomenclature based upon the prohormone structure is generally possible and preferred.

Human pre-proglucagon is a polypeptide of 179 amino acid residues that is proteolytically cleaved by a signal peptidase at the site of the Gln^{20}-Arg^{21} bond [1]. Amino acid residues in the signal peptide region are assigned negative numbers and the first (N-terminal) residue in the proglucagon sequence is designated +1. Consequently, the amino acids in the region of the site of cleavage of the signal peptide are designated: Ser^{-3}-Trp^{-2}-Gln^{-1}-Arg^{1}-Ser^{2}-Leu^{3}. All proglucagon-derived fragments are described by the residue number in proglucagon of their N- and C-terminal amino acids. The systematic and trivial names of the major proglucagon-derived peptides present in tissues are compared in Figure 1 and their locations within the proglucagon molecule are shown schematically. Although the systematic nomenclature is precise, it is somewhat unwieldy for repetitive use so that a strong case may be made for the use of a trivial name, providing that the systematic name is referred to first. Thus, proglucagon (1–69) becomes glicentin or enteroglucagon and the name insulinotropin has been proposed for proglucagon (78–108) [9].

Biosynthetic relationships between proglucagon-derived peptides

The human genome probably contains only a single copy of the pre-proglucagon gene that comprises five introns and six exons [1, 10]. However, discrepancies between the nucleotide sequences of the two reported structures of the cloned pre-proglucagon gene suggest the possibility of different alleles. Structural analysis

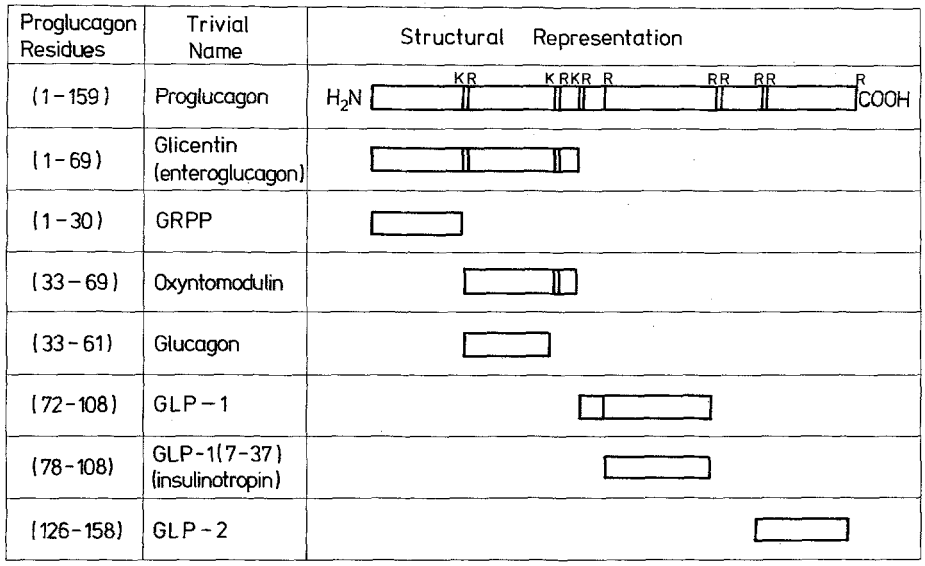

Fig. 1. A schematic representation of human proglucagon and the principal products of post-translational processing of proglucagon. GRPP: glicentin-related pancreatic peptide; GLP: glucagon-like peptide, K: lysine, R: arginine. The structure of proglucagon shown is predicted from the nucleotide sequence of the gene according to [1]. The structure predicted from the nucleotide sequence in [10] contains an additional C-terminal lysine residue

has shown that pre-proglucagon mRNAs in the pancreas, ileum and colon are identical [11, 12] and an identical transcriptional start site is used in the three tissues [12]. It has been concluded, therefore, that the diversity of the molecular forms of the proglucagon-derived peptides in the pancreas and gut arise from different pathways of post-translational processing of the primary transcript [11]. As shown in Figure 1, human proglucagon contains several pairs of dibasic amino acid residues (Lys-Arg and Arg-Arg) which can serve as potential recognition sites for a processing enzyme [13]. In the A-cell of the pancreas, the prohormone is processed to proglucagon (1-30), which is also referred to as glicentin-related pancreatic peptide (GRPP) [14], glucagon, the hexapeptide proglucagon (64-69) [15] but the remaining C-terminal fragment proglucagon (72-158) is not processed further [16]. In the L-cell of the gut, different dibasic residue cleavage sites are used so that proglucagon is processed to proglucagon (1-69) (glicentin) [17], proglucagon (33-69), which has been termed oxyntomodulin [18], GLP-1 (proglucagon (72-108)), an intervening peptide sequence (proglucagon (111-123)) and GLP-2 (proglucagon (126-158) [11, 19]. GLP-1 is a substrate for a second proteolytic enzyme in the gut whose specificity involves cleavage at the site of single arginine residues [20]. Consequently, the predominant molecular form of GLP in the intestine of the pig [21], rat [11] and humans [22] is the truncated peptide, GLP-1 (7-37). The predicted amino acid sequences of GLP-1 (7-37) and proglucagon (111-123) terminate in a glycine residue, so that there is a strong probability that the peptides contain an α-amidated C-terminal residue. Experimental confirmation of this hypothesis is required. Similarly, the predicted GLP-2 sequence terminates in an arginine residue, so that, by analogy with other systems [23], it is probable that this residue is removed by the action of an endogenous carboxypeptidase B-like enzyme, e.g. carboxypeptidase H. Chromatographic evidence has been provided to show that the glucagon-like peptide formerly described as IRG^{9000} probably represents proglucagon (1-61) [24]. This component is found in low concentration in the pancreas, gut and plasma of healthy subjects but is elevated in the plasma of uraemic patients.

The factors regulating the tissue-specific post-translational processing of proglucagon are not understood. In rat islets, an unusual 0-glycosidic glycosylation of the prohormone takes place early in the biosynthetic process [25]. It has been shown that purified porcine glicentin is a substrate for cyclic-AMP-dependent protein kinase [26]. Thus, it is tempting to speculate that such post-translational modifications to individual amino acids may be tissue-specific and alter the susceptibility of processing sites towards the cleavage enzyme(s).

Physiological roles of the proglucagon-derived peptides

The biological significance of peptides derived from the N-terminal region of proglucagon remains unclear. Proglucagon (1-69) (glicentin) [27], glicentin-related pancreatic polypeptide [28], oxyntomodulin [29] and the C-terminal octapeptide of oxyntomodulin [29] will inhibit, with varying degrees of potency and effectiveness, pentagastrin-stimulated gastric acid secretion in the rat. Oxyntomodulin (proglucagon (33-69)) is only one-tenth as potent as glucagon in stimulating adenylate cyclase in rat liver plasma membranes and one-fifth as potent in potentiating insulin-release from the perfused rat pancreas; but the peptide is 20-fold more potent than glucagon in inhibiting gastric acid secretion [30]. Nevertheless, a clear demonstration that the enterogastrone effect of proglucagon-derived peptides is physiologically relevant in man is needed. The asser-

tion that proglucagon (1-69) (enteroglucagon) is a trophic factor for the intestinal mucosa is based upon indirect evidence, such as the demonstration that plasma enteroglucagon levels correlate well with crypt cell production rate in several animal models of mucosal hypertrophy [31]. It has been shown, however, that continuous in vivo immunoneutralisation of enteroglucagon using a monoclonal antibody directed against the N-terminal to the central region of glucagon did not modify the adaptive response of the ileal remnant in rats with proximal small bowel resection [32]. The lack of availability of pure proglucagon (1-69) prevents the carrying out of definitive in vitro experiments.

Although structural similarity between the GLPs and glucagon is only moderate, the amino acid sequences of the GLPs themselves have been very strongly conserved during evolution [33]. This would suggest that the peptides are biologically important rather than merely "spacer" material in the precursor. The first attempts to determine the biological activity of GLP were performed using GLP-1 (1-37) or GLP-1 (1-36) amide. GLP-1 (1-36) amide showed only weak stimulatory activity and GLP-2 was without effect upon insulin release from isolated rat islets [33]. Similarly, GLP (1-37) was without effect on insulin release from the perfused rat pancreas in concentrations as high as 500 nmol/l [9]. Unprocessed GLP-1 does not increase hepatic glucose production in the rat [34] and the peptide given in high doses to ob/ob mice produced only a very small increase in plasma insulin concentrations and no change in plasma glucose [35]. In contrast, unprocessed GLP-1 binds to specific receptors in rat brain [36] and both GLP-1 and GLP-2 are potent stimulators of adenylate cyclase in rat hypothalamus and pituitary [37]. The distribution of GLP-1-like immunoreactivity in the brain is similar to that of glucagon-like immunoreactivity [36] and it has been suggested that these proglucagon-derived peptides may be important as modulators of neurotransmission.

An incretin is defined as a substance produced by the gut that is released into the circulation in response to nutrients and stimulates insulin release at physiologically relevant concentrations [38]. The evidence that the truncated form of GLP-1 (GLP-1 (7-36) amide and/or GLP-1 (7-37)) is a physiologically important incretin is strong. Studies in man have demonstrated that concentrations of GLP-1 (7-36) amide-like immunoreactivity rise after oral glucose and a test breakfast [22]. Infusion of the peptide at a rate that results in an increase in concentration that mimics the post-prandial rise results in significant rises in insulin concentrations and falls in plasma glucose [22]. Studies in vitro have shown that GLP-1 (7-37) at concentrations as low as 50 pmol/l will increase insulin release from isolated perfused rat pancreas [9]. Studies with cells derived from a transplantable rat insulinoma (RINm5F cells) have identified high affinity binding sites for GLP-1 (7-36) amide [39] and chemical cross-linking experiments have identified a binding protein for the peptide in the plasma membrane of mol.wt.63,000 (R.Göke and J.M.Conlon, unpublished data). Binding of GLP-1 (7-36) amide to the cells resulted in an increase in the concentration of cyclic AMP [39, 40] and stimulated insulin gene expression [40]. GLP-1 (1-36) amide bound to the cells with an affinity that was approximately 200-fold less than that of the truncated peptide. Exogenous glucagon did not significantly influence the release of GLP-1-like immunoreactivity from isolated perfused rat pancreas [41] but further studies are necessary to determine whether proglucagon-derived peptides regulate their own secretion. Specific binding sites for GLP-1 (7-36) amide on dispersed enterocytes were not detected, which suggests, but does not prove that the peptide is not a growth factor for intestinal mucosa [39]. Clearly, further studies are warranted to examine the roles of the GLPs in the pathophysiology of diabetes and gastrointestinal and metabolic diseases. Preliminary work [22] has implicated hypersecretion of the truncated form of GLP-1 in the hyperinsulinaemia and reactive hypoglycaemia of postgastrectomy dumping syndrome.

The author of a previous review of the subject in the journal [7] considered it prudent to include a question mark in the title "The glucagon-like polypeptides - order out of chaos?" Perhaps, the question mark may now be safely omitted.

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Dr. J.M.Conlon
Klinische Arbeitsgruppe der MPG
Gosslerstraße 10D
D-3400 Göttingen
FRG