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REVIEW

Glucagon-like peptide-1(7–36) amide is a new incretin/enterogastrone candidate

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Introduction

Cloning and sequence analysis of cDNAs and DNA fragments from genomic libraries has led to a dramatic increase in understanding of the glucagon-related peptides in recent years. The primary structure of the biosynthetic precursor of glucagon (preproglucagon) has been elucidated. The complex structural connections between the multiple molecular forms of the glucagon-like peptides in tissues and in the circulation have been determined [1,2].

Mammalian proglucagon consists of 160 amino acid residues and is synthesized in the islets of Langerhans, intestine and brain. An exciting recent finding is that in addition to glucagon the preproglucagon gene in mammals encodes two additional peptides with structural similarity to glucagon, termed glucagon-like peptide-1 and -2 (GLP-1 and GLP-2). The truncated form of GLP-1, GLP-1(7–36) amide, which is secreted by the mammalian intestine, strongly stimulates insulin secretion and inhibits gastric acid secretion. This review focuses mainly on the truncated form of GLP-1, since a rapidly increasing number of reports indicate a remarkable interest of investigators in this particular hormone, which actually fulfills the classical role of an 'enterogastrone' and 'incretin' hormone. The purpose here is to describe what is known so far about the origin, processing, organ distribution and actions of this peptide and to search for promising future directions of further investigations.

Origin and processing of pre-proglucagon-derived peptides

Probably only a single copy of the pre-proglucagon gene is contained in the human genome. This gene comprises five introns and six exons [3,4,5]. It has been shown that preproglucagon messenger RNA from pancreas and intestine are identical [6,7] but the pathway of post-translational processing of the primary transcript differs markedly in the two tissues [1]. Mammalian proglucagon exhibits several pairs of dibasic amino acid residues which are potential processing sites for enzymes [8]. In the A-cell of the pancreas proglucagon is predominantly processed into proglucagon(1–30) (also called glicentin-related pancreatic peptide (GRPP) [9]), proglucagon(33–61) which is identical with glucagon, the hexapeptide proglucagon(64–69) [10] and the carboxy-terminal fragment proglucagon(72–158) [11]. The carboxy-terminal fragment contains the sequences of glucagon-like peptide-1 and glucagon-like peptide-2 but is not further processed in the pancreas [11] (Fig. 1).

In the L-cell of the gut proglucagon is predominantly processed to proglucagon(1–69) also called glicentin [12], oxyntomodulin (proglucagon(33–69)) [13], glucagon-like peptide-1 (GLP-1; proglucagon (72–108)) and glucagon-like peptide-2 (GLP-2; proglucagon(126–158)) [6,14]. GLP-1(1–37) is further processed to GLP-1(7–37) [1] which is the predominant form of GLP-1 in the gut of human [14], rat [6,16] and pig [17].

Recently, it was demonstrated that the truncated form of pig and human GLP-1 contains an α-amidated carboxy-terminal amino acid residue [15]. It is interesting that the amino acid sequence of GLP-1 closely resembles that of glucagon; 14 of 19 amino acid residues are identical to glucagon when position 7 (histidine) of GLP-1 is aligned with position 1 (histidine) of glucagon. It is believed that histidine at position 7 of GLP-1 as the free N-terminal amino acid is of special importance in GLP-1's insulinotropic activity and probably in its glucagon-inhibiting activity, and that C-terminal amino acids are less important for these effects [18,19].

The sequence of GLP-1 is identical in various different mammals like rat, hamster, guinea-pig, bovine and man [1,2,20]. Such strong conservation of the peptide sequence during evolution points to an important biological role for GLP-1.

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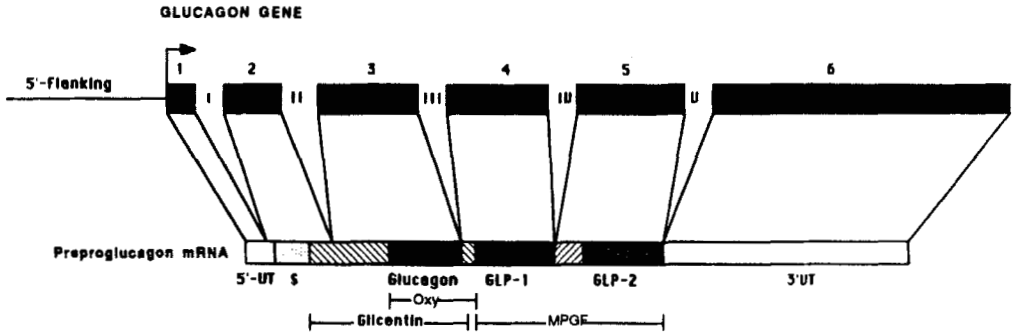

Figure 1. Schematic figure of the glucagon gene with its six exons (1–6) and the proglucagon mRNA containing the proglucagon derived peptides. 5'-UT, 5'-untranslated sequences; 3'-UT, 3'-untranslated sequences; S, signal peptide; GLP-1, glucagon-like peptide-1; GLP-2, glucagon-like peptide-2; MPGF, major proglucagon fragment; Oxy, oxyntomodulin (from Drucker DJ. Pancreas 1990;5:484–488).

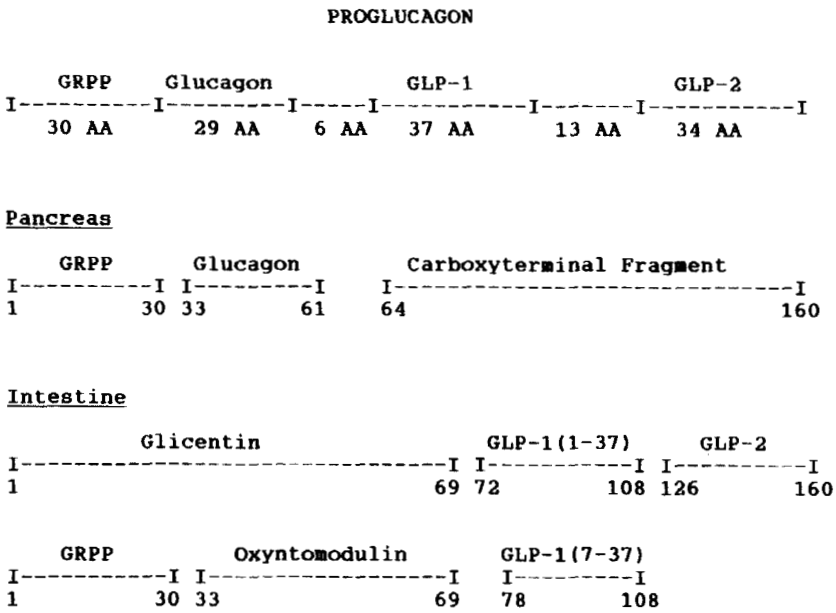

Figure 2. Tissue specific processing of proglucagon in pancreas and intestine (AA = amino acid).

Specific receptors for GLP-1(7–36) amide

The discovery that insulin-producing cells contain specific binding sites for GLP-1(7–36) amide supports the assumption that the truncated form of GLP-1 may be a gastrointestinal hormone of physiological significance [21]. Whereas no binding sites could be demonstrated on enterocytes from pig jejunum [21], highly specific binding of ^{125}I-labelled GLP-1(7–36) amide to rat insulinoma derived RINm5F cells was found [21] (Fig. 2). Scatchard analysis of the binding data revealed the presence of a single class of binding sites with a dissociation constant (Kd) of $2{\cdot}04 \times 10^{-10}$ M. In cross-linking studies with RINm5F cell membranes the molecular weight of the binding protein for GLP-1(7–36) amide was determined to be 63 000 [22]. Since no change in the mobility of the receptor band was observed under reducing conditions it was suggested that the binding protein in the receptor is not attached to other subunits via disulphide bonds [22].

Binding of the truncated GLP-1(7–36) amide resulted in a dose-dependent increase of cAMP production [21], which suggests that the binding site is a receptor linked to a functional response and not, for example, a site linked to a system of proteolytic inactivation. Binding of GLP-1(7–36) amide to RINm5F cell membranes was decreased by the introduction of guanine nucleotides to the assay system. This effect was due to a decrease of the receptor affinity, whereas the receptor number remained unchanged [23]. In contrast to fuel and non-fuel secretagogues, GLP-1(7–36) amide did not cause a depolarization of membrane potential or changes in cytosolic free calcium levels in RINm5F cells [23]. These data suggest that the action of the peptide is mediated by the adenylate cyclase system. This assumption is supported by the finding that GLP-1(7–37) also increased cAMP levels in another insulin-producing cell line [24]. An involvement of the inositol 1,4,5-trisphosphate pathway or an activation of pro-

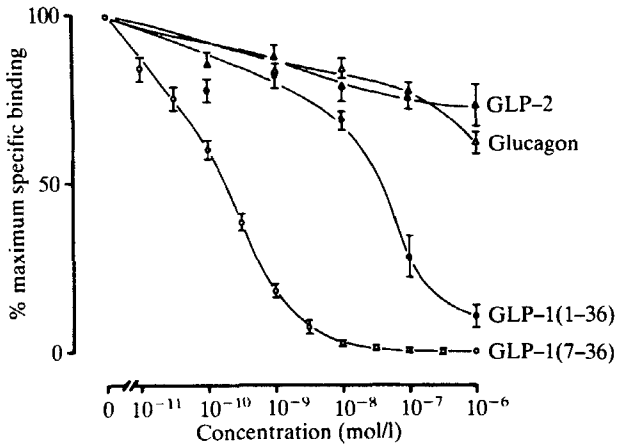

Figure 3. Inhibition of binding of ^{125}I-labelled glucagon-like peptide-1(7-36) amide to rat RINm5F cells by GLP-1(7-36) amide, GLP-1 (1-36) amide, glucagon and GLP-2. Incubations were carried out for 30 min at 37°C at a cell concentration equivalent to 20 μg DNA/tube. Data points show means ± SEM for at least six experiments. Specific binding is defined as the total binding of radioactivity minus the binding in the presence of 1 μmol GLP-1(7-36) amide/l (from Göke R, Conlon JM. J Endocrinol 1988;116:357-362).

tein kinase C in the post-receptor signalling after GLP-1(7-36) amide binding appears to be unlikely.

In a recent study, receptors for GLP-1(7-37) have been identified on isolated rat gastric glands [25]. Scatchard analysis revealed the presence of a single class of binding sites with a Kd of $4{\cdot}4 \times 10^{-10}$ M. Binding of truncated GLP-1 resulted in an increase of cAMP formation with an EC_{50} of $1{\cdot}6 \times 10^{-10}$ M. These data demonstrate that the receptors on gastric glands are similar to the receptors which were identified on rat insulinoma cells. Recently, we characterized receptors for GLP-1(7-36) amide on rat lung membranes [26]. Scatchard analysis showed the presence of a single class of binding sites with a Kd of $1{\cdot}67 \times 10^{-9}$ M. The binding affinity was 10 times less compared to the receptors on RINm5F cells ($1{\cdot}67 \times 10^{-9}$ M *vs* $0{\cdot}204 \times 10^{-9}$ M). In contrast to our results with RINm5F cells, we found that VIP and PHI were more potent in displacing ^{125}I-labelled GLP-1(7-36) amide from the rat lung receptors than other proglucagon-derived peptides except GLP-1(7-36) amide itself. The order of potency was GLP-1(7-36) amide > VIP > PHI > GLP-1(1-36) amide > glucagon and GLP-2 [26]. Binding of ^{125}I-labelled GLP-1(7-36) amide to rat lung receptors was inhibited by guanine nucleotides, suggesting that these receptors similarly to GLP-1 (7-36) amide receptors on RINm5F cells and gastric glands are coupled to the adenylate cyclase system [26].

Additional data have been published which demonstrate the existence of high affinity receptors for GLP-1(7-36) amide in rat brain [27]. Again, binding of the peptide resulted in an increased cAMP production [27].

Up to now, the receptor for GLP-1(7-36) amide has not been purified to an extent which would allow sequencing and eventually cloning. It would be important to raise specific antibodies against the receptor protein, which would, for example, allow *in vivo* immunoneutralization studies to prove the physiological effects of the peptide.

Internalization of GLP-1(7-36) amide

Receptor-mediated endocytosis appears to be a general mechanism by which cells internalize hormones after initial cell-surface binding. It was demonstrated that after binding to RINm5F cells GLP-1(7-36) amide is internalized and degraded intracellularly. Chloroquine was shown to inhibit the intracellular degradation and to delay the release of the degradation products [28] suggesting that the cleavage of the internalized peptide is facilitated by lysosomal degradation. It remains to be elucidated whether GLP-1(7-36) amide or a degradation product of the peptide has any effect on intracellular metabolism. Furthermore, it has not yet been proven whether the GLP-1-receptor undergoes internalization and consecutive degradation or recycling, another reason to generate antibodies which are directed against receptor protein.

GLP-1 and endocrine pancreas

(1) GLP-1-like immunoreactivity (IR) in the pancreas

GLP-1-like IR was identified in the pancreas by radioimmunoassay and immunocytochemistry using polyclonal antibodies against GLP-1(1-19) [29,30], GLP-1(1-37)/(1-36) amide [28-32] and monoclonal antibodies against GLP-1(1-36) amide [32]. GLP-1-like IR coexists with glucagon-like IR in the marginal zone of pancreatic A cells [29,30,32,33]. Analysis of pancreatic extracts showed that in human and pig pancreas most of the GLP-1-like IR has a molecular weight of 10 kDa [14,29,31]. This is in accordance with Patzelt & Schiltz, who reported preproglucagon to be processed to glucagon and a carboxyterminal fragment of 10 kDa molecular weight [11]. In gel filtration of rat pancreatic extracts Manaka *et al.* [32] identified the major peak with a molecular weight of 4·2 kDa in a position identical to synthetic GLP-1(1-37) as has similarly been shown in cattle in a previous study [36]. This difference is perhaps due to species-specific proglucagon processing in the pancreas.

(2) GLP-1-like immunoreactivity (IR) released from the pancreas

GLP-1-like IR is co-released with glucagon-like IR from the pancreas in response to arginine stimulation. In human and pig pancreas the majority of the GLP-1-like IR released upon arginine stimulation has an MW of 10 kDa while in rat pancreas the 4·2-kDa form predominates [14,32,36,37]. Although some GLP-1-like material is released from the endocrine pancreas by far the most originates from the gut.

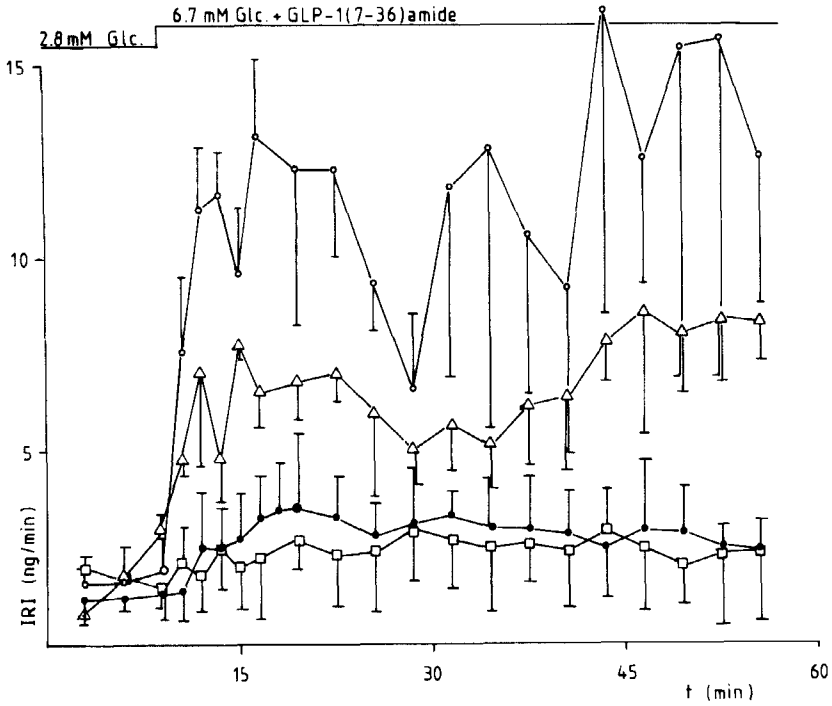

Figure 4. Stimulation of insulin release from perfused rat pancreas by GLP-1(7–36) amide. Isolated pancreas was perfused in the absence (□) and presence of 0·05 nM (●, 0·5 nM (△), and 5 nM (○) GLP-1(7–36) amide in the perfusate. Data points show means ± SD of six experiments (from Göke R, Fehmann HC, Richter G, Trautmann M, Göke B. Pancreas 1989;4:668–673).

(3) Effects of GLP-1 on the endocrine pancreas

Initial studies did not demonstrate any biological activity of the peptide. GLP-1(1–36) amide did not alter blood glucose or insulin levels in fasted canines [38]. Schmidt, Siegel & Creutzfeldt obtained a weak stimulatory effect on insulin secretion in isolated rat pancreatic islets using supraphysiological concentrations of GLP-1(1–36) amide [39], while GLP-1(1–36) amide had no insulinotropic effect in the isolated perfused pig pancreas as shown by Orskov, Holst, Knuhtsen, Baldissera, Poulsen & Vagn Nielsen [14]. In obese hyperglycaemic mice, Bailey & Flatt found a very small insulin-releasing effect of GLP-1(1–37) with no change of the blood glucose levels (40).

A 'break-through' in this discussion about a possible physiological role for GLP-1 was achieved by Drucker, Philippe, Mojsov, Chick & Habener in a later study which reported that not GLP-1(1–37), but the truncated form of this peptide potently increases cAMP levels, insulin mRNA transcripts, and insulin release in cultured rat insulinoma cells (RIN 1046–36). These findings were the first indication that the truncated peptide, GLP-1(7–36) amide, is the biologically active form [24]. This assumption was confirmed by others who demonstrated a strong insulinotropic effect of GLP-1(7–37) and GLP-1(7–36) amide which were equipotent [17,41].

In the isolated perfused pig pancreas in the presence of 7 mM glucose, GLP-1(7–36) amide at a concentration of 10^{-10} M induced a 1·4-fold increase, and at 10^{-9} M a 2·3-fold increase of insulin release. Arterial infusion and adjustment to a level of 10^{-10} M GLP-1 (7–37), resulted in an 2-fold increase, whereas a 10^{-9} M concentration resulted in a 4-fold increase of the insulin release [17]. In the isolated perfused rat pancreas in the presence of 6·6 mM glucose, GLP-1(7–37) at a concentration of 5×10^{-11} M led to a 3–10-fold elevation of insulin secretion [41]. No effect on insulin secretion was found with GLP-1(1–37). Additional studies confirmed these observations showing a strong insulinotropic effect of GLP-1(7–36) amide on insulin secretion [42,43,44,45] (Fig. 3). Studies in man demonstrated that circulating GLP-1(7–36) amide levels rose after oral glucose and after a meal. Infusion of the peptide in doses mimicking post-prandial concentrations resulted in a significant rise in insulin concentrations and a fall in blood glucose [46]. In this context it is of interest to mention that for a similar effect on glucose-stimulated insulin secretion the effective concentration of GLP-1(7–36) amide was reported to be as low as one-tenth of that of GIP [43], the strongest incretin candidate up to recently, which indicates that GLP-1(7–36) amide has a greater insulinotropic effect than GIP. Taken together, GLP-1(7–36) amide fulfills the classic conditions that allow its identification as an incretin hormone [47,48]: it is released from the gut by nutrients, especially carbohydrates, and it stimulates insulin secretion in the presence of elevated blood glucose levels.

Studies in man revealed that GLP-1(7–36) amide has not only an insulinotropic but also a glucagon-

suppressing effect [46]. This was confirmed by experiments with isolated perfused pig pancreas in which GLP-1(7–36) amide at a concentration of 10^{-10} M inhibited glucagon secretion by 50% and at 10^{-9} M by 70–80% [42]. Similar results were obtained in studies with isolated perfused rat and canine pancreas [49,50].

Truncated GLP-1(7–36) amide significantly increased somatostatin secretion from the isolated perfused pig pancreas. At 10^{-9} M GLP-1(7–36) amide somatostatin release was more than doubled [42] compared to controls. At present it is unclear whether the glucagon-suppressing effect of the truncated GLP-1 is a direct result of the peptide or whether this effect is mediated by somatostatin, since somatostatin was reported to be a mediator of the suppression of glucagon during hyperglycaemia [51]. This latter explanation is supported by a recent report by D'Alessio, Fujimoto & Ensick which demonstrated in islet cell monolayer cultures from rat pancreas that GLP-1(7–36) amide increased somatostatin release by 40% but left glucagon release unaffected [52]. It is possible that in intact islets the paracrine effects of somatostatin and insulin may inhibit glucagon release after GLP-1(7–36) amide exposure.

Recent studies compared the effects of various N-terminal and C-terminal fragment peptides of GLP-1(7–36) amide on insulin and glucagon release from the isolated perfused rat pancreas as well as on cAMP formation in the βTC1 cell line [18,19]. Concerning the N-terminal portion, GLP-1(7–37) amide was proved to be a potent insulinotropic secretagogue while GLP-1(1–37) amide, GLP-1(6–37) amide and GLP-1(8–37) amide showed no effect on insulin release. Concerning the C-terminal portion, GLP-1 (7–37) amide, GLP-1(7–36) amide and GLP-1(7–37) showed similar potency in stimulating the insulin release while GLP-1(7–34) and GLP-1(7–35) were less potent and GLP-1(7–20) and GLP-1(7–33) had no effect. Similar results were found when the cAMP formation in the βTC1 cell line was studied. Glucagon release was suppressed by GLP-1(7–37), GLP-1(7–37) amide and GLP-1(7–36) amide at concentrations in which these peptides stimulated insulin release (1 and 10 nM) while other fragments had no effect. These results suggest that histidine at position 7 of GLP-1 (7–37) is important for the stimulation of cAMP production, insulin release, and inhibition of glucagon release. Furthermore, the three C-terminal amino acids as well as the C-terminal amidation seem to be less important for these effects. It would be of interest to employ a sensitive and specific antibody against GLP-1(7–36) amide to induce an experimental GLP-1(7–36) amide deficiency *in vivo*. This could help to determine the importance of the hormone in the control of insulin secretion. Unfortunately, a first approach using an antibody against the precursor molecule GLP-1 revealed rather ambiguous results [53]. Whereas the insulinotropic effect of exogenous GLP-1(7–36) amide was reduced, injection of GLP-1 antiserum did not decrease insulin release after an oral glucose load in rats [53]. These experiments have to be repeated using specific antibodies directed against the truncated form of the peptide.

(4) Interaction of GLP-1(7–36) amide with other hormones

Accumulating evidence suggests that several intestinal hormones are involved in the regulation of the entero-insulinar-axis [48]. Furthermore, it is likely that various incretin factors act in concert to modulate insulin release. Zawalich reported that a combination of GIP and cholecystokinin (CCK) leads to a markedly amplified glucose-induced insulin response in perifused rat islets [54] and Ahren, Hedner & Lundquist demonstrated a strong insulinotropic effect of GIP and CCK-8 injected together in low doses in mice [55]. From these findings, it was of interest to study the interaction of GLP-1(7–36) amide, CCK-8 and GIP on insulin secretion. The GLP-1(7–36) amide (5×10^{-10} M) stimulated, glucose-induced (6·7 mM) insulin release from the perfused rat pancreas was potentiated by CCK-8 (maximal effect at 5×10^{-11} M) [56] (Fig. 4). GLP-1(7–36) amide (5×10^{-10} M) and GIP (maximal effect at 10^{-9} M) exerted an additive synergistic effect upon glucose-induced insulin release [57] (Fig. 4). Since the effect of GLP-1(7–36) amide is mediated by the adenylate cyclase system [23] the potentiation of the GLP-1 effect by CCK was explained by an interaction of different second messenger systems [56] as was suggested in the case of GIP and CCK [54]. Since both GLP-1(7–36) amide and GIP act via the adenylate cyclase system a combination of both revealed an additive synergistic effect which was only obtained when submaximal effective hormone concentrations were utilized [57].

Another exciting possibility for an interaction of gastrointestinal hormones in regulating insulin secretion was reported from experiments in which different incretin candidates were investigated for their ability to sensitize the endocrine pancreas against subsequent stimulation by another stimulus. A short lasting preinfusion of GLP-1(7–36) amide, GIP, and CCK induced an augmented insulin secretion in response to subsequent glucose stimulation [58].

It has been shown that the GLP-1(7–36) amide stimulated cAMP production in RINm5F cells is partially inhibited by somatostatin-14 [45]. Furthermore, somatostatin lowered the glucose-induced, GLP-1(7–36) amide potentiated insulin secretion [46]. The intracellular mechanism of this inhibitory action of somatostatin on the insulin release has yet not been fully explained. However, due to the observation that somatostatin inhibits secretagogue-stimulated cAMP accumulation in the exocrine pancreas [59] it seems likely that the inhibitory action of somatostatin on the glucose-induced, GLP-1-potentiated insulin release is at least partly related to a regulation of the adenylate cyclase system.

It has been demonstrated that in RINm5F cells,

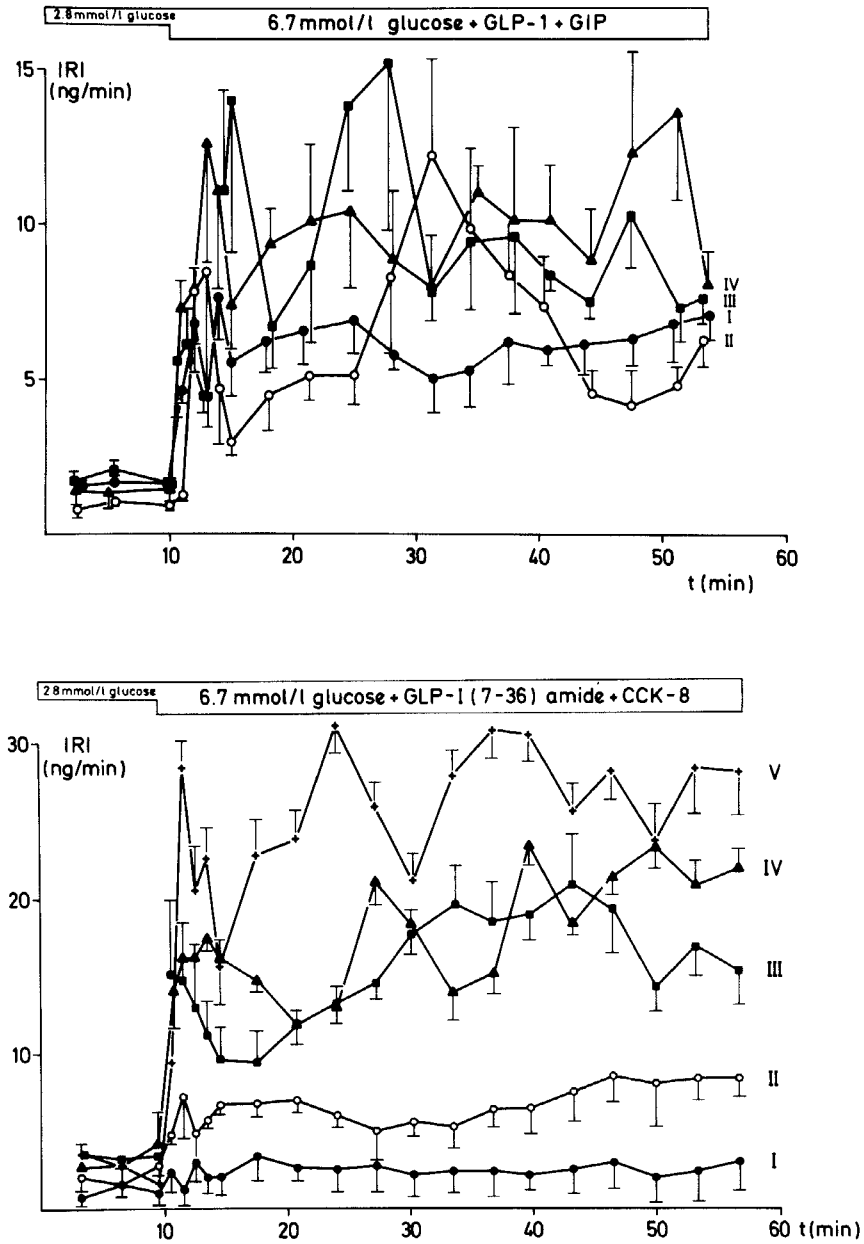

Figure 5. Effect of gastric inhibitory peptide (GIP) (upper panel) and cholecystokinin-8 (CCK-8) (lower panel) on glucose (6·7 mM)-induced and GLP-1(7–36) amide (0·5 nM)-potentiated insulin secretion from the isolated perfused rat pancreas. Upper panel: (I) glucose + GLP-1(7–36) amide; (II) glucose + GLP-1(7–36) amide + GIP (0·1 nM); (III) glucose + GLP-1 (7–36) amide + GIP (1 mM); (IV) glucose + GLP-1(7–36) amide + GIP (10 nM) (from Fehmann HC, Göke B, Göke R, Trautmann ME, Arnold R. FEBS Lett 1989;252:109–112. Lower panel: (I) glucose; (II) glucose + GLP-1(7–36) amide; (III) glucose + GLP-1(7–36) amide + CCK-8 (20 pM); (IV) glucose + GLP-1(7–36) amide + CCK-8 (100 pM); (V) glucose + GLP-1(7–36) amide + CCK-8 (50 pM) (from Fehmann HC, Göke B, Weber V, Göke R, Trautmann ME, Richter G, Arnold R. Pancreas 1990;5:361–365). Data points show means ± SEM of at least six experiments.

pretreated with dexamethasone for up to 72 h, binding of GLP-1(7–36) amide was markedly reduced. Further analysis of the binding data revealed a reduction of the receptor number while the receptor affinity remained unchanged [60]. From these results it was speculated that the effect of GLP-1(7–36) amide receptors induced by glucocorticoids could contribute to the impaired glucose tolerance in glucocorticoid-induced diabetes [60]. However, this suggestion remains rather speculative and needs further confirmation.

Up to now, clinical studies considering physiological and pathophysiological implications of GLP-1(7–36) amide are clearly lacking. Although initial human studies [46] indicated that GLP-1(7–36) amide is a physiological incretin in man, these findings have to be corroborated and extended to patients suffering from different forms of diabetes mellitus before clear conclusions can be drawn. Results are expected in the near

future since several groups are currently developing or validating sensitive and reliable radioimmunoassay systems.

GLP-1 and the exocrine pancreas

Studies with isolated rat pancreatic acini showed that neither GLP-1(7–36) amide [56] nor any of the proglucagon-derived peptides [61] have an effect on basal and cholecystokinin-stimulated amylase release from isolated rat acini. No information is available on the effect on pancreatic secretion under other conditions.

GLP-1 and liver

Searching for possible effects of GLP-1 on glucose metabolism Ghiglione *et al.* and Shimizu, Hirota, Ohboshi & Shima failed to demonstrate specific binding sites on rat liver membranes [62,63]. Furthermore, GLP-1(7–36) amide did not interact with glucagon receptors, in accordance to a previous study published by Hoosein & Gurd [64]. GLP-1(1–36) amide did not alter the cAMP level in the presence or absence of glucagon [62] and had no effect on glycogenolysis in cultured rat hepatocytes [63].

It is possible to explain the failure of GLP-1 to influence hepatic glucose metabolism by the fact that the precursor molecule GLP-1 was used instead of the truncated peptide. However, in studies with GLP-1 (7–36) amide no effect on glucose metabolism was found in the isolated perfused rat liver (KH Beckh, Marburg; personal communication). In contrast, fish GLPs were reported to stimulate glycogenolysis, gluconeogenesis and lipolysis [65–69]. These actions were found to be very variable depending on season and fish species (66–70). As known so far, preproglucagon-derived peptides in several species of elasmobranch, holostean and teleostean fishes contain only one GLP corresponding mainly to GLP-1(7–37) [66,71]. Interestingly, the activation of gluconeogenesis in these studies was not accompanied by an increase of cAMP formation. Since no specific binding sites were identified on liver membranes, the authors postulated the presence of intracellular recognition sites for GLPs [65]. However, there are no data available yet to prove this suggestion. Further studies are necessary to elucidate the action of GLP-1(7–36) amide on hepatic glucose metabolism.

GLP-1 and stomach

GLP-1(7–36) amide is a potent inhibitor of the pentagastrin-induced gastric acid secretion in man. In low concentrations, such as 30 pmol/l [72] and 60 pmol/l [73], the peptide reduced pentagastrin-stimulated gastric acid secretion by 36% [72] and almost 50% [73], respectively and volume secretion by 33 and 36% [73]. Incubation of rat gastric glands or human gastric cancer cells with GLP-1(7–36) amide resulted in strong stimulation of cAMP production [25,73]. This latter finding is surprising since the enhancement of cAMP has an important role in the stimulation of acid secretion [74]. Interestingly, a very recent study revealed the existence of a single class of high affinity receptors for GLP-1(7–36) amide in rat gastric glands which were coupled to the adenylate cyclase system [25]. As long as these experiments have not been repeated with pure parietal cells the question is open as to whether or not the inhibitory effect of GLP-1 on the pentagastrin-stimulated acid secretion is mediated by cAMP. For the inhibitory effect of GLP-1(7–36) amide on acid secretion, alternatively an indirect effect possibly mediated by a regulation of additional hormonal or paracrine mediators should be taken into account.

Eissele, Koop & Arnold recently demonstrated a stimulatory effect on somatostatin release and an inhibition of gastrin secretion by GLP-1(7–36) amide in the isolated perfused rat stomach suggesting that the GLP-1(7–36) amide-induced inhibition of gastric acid secretion may be mediated by an enhanced somatostatin and/or a lowered gastrin release [75]. On the other hand, in pigs GLP-1(7–36) amide had no effect on somatostatin or gastrin secretion from the antrum or on somatostatin secretion from the nonantral stomach [42], suggesting that the inhibitory effect of GLP-1 on gastric acid secretion does not involve alterations of the somatostatin or gastrin level. These contradictory results could be due to species-specific differences in the regulation of acid secretion. Clearly, more work is necessary to understand fully the action of truncated GLP-1 on the stomach. However, the effects documented up to now strongly argue in favour of an enterogastrone-like activity of this peptide.

GLP-1 and intestine

Using polyclonal antibodies against GLP-1(1–19) and GLP-1(1–37) GLP-1-like immunoreactivity was identified in colorectal enteroglucagon cells co-existing with glicentin-like immunoreactivity within single secretory granules [30,33]. Sequence determination of purified GLP-1 from rat, pig and human intestine revealed that the peptide is identical to proglucagon 78-107 amide [15,16]. Investigations on the distribution of GLP-1-like immunoreactivity in the gastrointestinal tract showed highest concentrations in the terminal ileum and colon [16]. The GLP-1(7–36) amide content of the colon is increased in rats with streptozotocin-induced diabetes, which showed an increased food intake [16,76]. A similar increase of glucagon-like immunoreactivity has been shown in hypothermia-induced hyperphagia rats [77], so that the higher concentrations of GLP-1-like- and glucagon-like-immunoreactivity in the colon could be a consequence of an increased food intake. In rats with streptozotocin-induced diabetes, plasma levels and ileal peptide concentrations of glucagon-like immunoreactivity were elevated on days 8–22 of diabetes [76]. Pancreatic

glucagon-like immunoreactivity showed only a transient rise on day 1 [76]. These differences between normal and diabetic rats were not due to an alteration of post-translational processing of proglucagon [76]. It has been speculated that peptides from proglucagon may exercise a trophic effect upon gastrointestinal mucosa [78]. However, no specific binding sites for ^{125}I-labelled GLP-1(7–36) amide could be detected on dispersed pig enterocytes [21]. This finding suggests, but does not prove, that the truncated form of GLP-1 is not a growth factor for the intestinal mucosa.

GLP-1 and central nervous system

GLP-1-like immunoreactivity has been identified in thalamus, hypothalamus, in the medullary nucleus tractus solitarius and in the dorsal and ventral parts of the medullary reticular nucleus [27,33,34,79–81]. While proglucagon-mRNA from pancreas and intestine is identical, Han, Hynes, Jin, Towle, Lauder & Lund [34] demonstrated the presence of two different proglucagon mRNAs in rat brain. Cells in the medullary nucleus of the solitary tract hybridized to a synthetic oligonucleotide probe corresponding to nucleotide sequences in pancreatic proglucagon mRNA encoding GLP-1, whereas cells in the hypothalamus did not. Polyadenylated RNAs from fetal rat brain contain two mRNAs. One mRNA of 1300 bases has the same size as pancreatic proglucagon mRNA while the second of 1500 bases may represent the hypothalamic proglucagon mRNA. These data strongly suggest the presence of a proglucagon mRNA in hypothalamus different from that in the pancreas and intestine [34]. However, studies with a rat pituitary tumour cell line (GH_4C_1), in which a metallothionein-glucagon fusion gene was introduced and expressed, showed that proglucagon is expressed in the brain in the same manner as in the intestine [82]. These results were confirmed by studies demonstrating the presence of GLP-1(7–36) amide in extracts of rat brain [27,79,81].

Furthermore, high affinity receptors for GLP-1(7–36) amide were identified in rat brain [27,83]. The distribution of receptors corresponds to the distribution of GLP-1-like immunoreactivity, with the exception of the pituitary gland. Thalamus, hypothalamus, pituitary gland and medulla oblongata were shown to be rich in GLP-1-binding sites [27]. Binding of the peptide resulted in an increased cAMP production in thalamus, hypothalamus and pituitary gland [27,84].

A recent study which determined the subcellular distribution of GLP-1-like immunoradioactivity revealed the presence of GLP-1(7–36) amide in the synaptosome fraction [80]. It has been shown that GLP-1(7–36) amide is released from hypothalamic tissue slices in a calcium-dependent manner by potassium stimulation [81]. It seems clear that GLP-1(7–36) amide has to be considered as a putative neurotransmitter.

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