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Glucagon-Like Peptide-1 (7–36) Amide as a Novel Neuropeptide

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Abstract

Although earlier studies indicated that GLP-1 (7–36) amide was an intestinal peptide with a potent effect on glucose-dependent insulin secretion, later on it was found that several biological effects of this peptide occur in the brain, rather than in peripheral tissues. Thus, proglucagon is expressed in pancreas, intestine, and brain, but post translational processing of the precursor yields different products in these organs, glucagon-like peptide-1 (7–36) amide being one of the forms produced in the brain. Also, GLP-1 receptor cDNA from human and rat brains has been cloned and sequenced, and the deduced amino acid sequences are the same as those found in pancreatic islets. Through these receptors, GLP-1 (7–36) amide from gut or brain sources induces its effects on the release of neurotransmitters from selective brain nuclei, the inhibition of gastric secretion and motility, the regulation of food and drink intake, thermoregulation, and arterial blood pressure. Central administration (icv) of GLP-1 (7–36) amide produces a marked reduction in food and water intake, and the colocalization of the GLP-1 receptor, GLUT-2, and glucokinase mRNAs in hypothalamic neurons involved in glucose sensing suggests that these cells may be involved in the transduction of signals needed to produce a state of fullness. In addition, GLP-1 (7–36) amide inhibits gastric acid secretion and gastric emptying, but these effects are not found in vagotomized subjects, suggesting a centrally mediated effect. Similar results have been found with the action of this peptide on arterial blood pressure and heart rate in rats. Synthesis of GLP-1 (7–36) amide and its own receptors in the brain together with its abovementioned central physiological effects imply that this peptide may be considered a neuropeptide. Also, the presence of GLP-1 (7–36) amide in the synaptosome fraction and its calcium-dependent release by potassium stimulation, suggest that the peptide may act as a neurotransmitter although further electrophysiological and ultrastructural studies are needed to confirm this possibility.

Index Entries: GLP-1 (7–36) amide; brain; brain receptors; central effects; neurotransmitter release; food intake; water intake; thermoregulation; gastric acid secretion; arterial blood pressure.

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Introduction

Glucagon or "glucose-driving" was the term used in 1923 by Kimball and Murlin to name the hyperglycemic factor isolated from pancreatic extracts, which they suggested might be the second hormone from the islets of Langerhans with an important role in the control of glycemia. One year before, Banting and Best (1922) had observed that after iv administration of pancreatic extracts, a rapid increase in blood glucose occurred before the hypoglycemic effect resulting from insulin. Despite these pioneer findings, the existence of this hyperglycemic factor was forgotten over the following 40 years, until crystalline glucagon was obtained. This allowed workers to know its primary structure, as well as its biological activities. Later on, the development of a radioimmunoassay (RIA) for glucagon determinations offered a considerable advance for studies related to the synthesis and release of this hormone, whereas application of recombinant DNA technology has shown glucagon to be a member of the family known as "glucagon and related peptides."

Glucagon and Related Peptides: The Structure of Proglucagon

The use in the RIA technique of specific antisera against pancreatic glucagon and of nonspecific ones that recognize both glucagon and a family of peptides of intestinal origin with properties similar to those of glucagon gave rise to the nomination of (GLI) glucagon-like immunoreactivity. At present, we know that GLI peptides are included in the molecule of preproglucagon (Fig. 1), which is produced in the pancreas, stomach, gut, and central nervous system (CNS).

The application of recombinant DNA technology led to the elucidation of the primary structure of proglucagon (Bell et al., 1983) from a variety of species, including humans. This has permitted interpretation of the structural relationships among the multiple forms (Mojsov et al., 1986) of glucagon-containing (glicentin and oxyntomodulin) and glucagon-like peptides (GLP-1 and GLP-2). A similar structure of the proglucagon gene, with six exons and five introns (Heinrich et al., 1984) has been reported in humans, rat, hamster, and guinea pig. This gives rise to an mRNA transcript that is identical in sequence in the pancreas, intestine, and brain, although posttranslational processing of the precursor yields different products in these organs (Mojsov et al., 1986). Beginning at the N-terminal portion preproglucagon (Fig. 1) contains a signal peptide of 20 amino acids, the sequences containing glucagon (glicentin and oxyntomodulin), and then (GLP-1 and GLP-2). In the A-cells of the pancreas, the products are glicentin-related pancreatic peptide (GRPP), glucagon, and a large peptide containing the sequences for GLP-1 and GLP-2. In the L-cells of the gut, proglucagon is predominantly processed to glicentin, oxyntomodulin, GLP-1, and GLP-2. Further processing of GLP-1 in these cells (Fig. 1) produces the truncated and amidated forms of the peptide: GLP-1 (1–36) amide, GLP-1 (7–36) amide, and GLP-1 (7–37). In the brain, the processing of proglucagon resembles that seen in the intestine.

Glucagon-Like Peptides (GLPs)

The C-terminal portion of mammalian proglucagon, which contains the sequences of GLP-1 and GLP-2, is proteolytically processed in intestinal endocrine L-cells and is released to the blood circulation as GLP-1 (1–37), GLP-1 (7–37), GLP-1 (7–36) amide, and GLP-2. The secretion of these peptides increases in response to the ingestion of ordinary mixed meals or oral glucose (Orskov et al., 1994). The truncated forms of GLP-1 are the most potent agents known to produce, a glucose-dependent stimulus for insulin release (Mojsov et al., 1987; Kreymann et al., 1987) and, within the entero–insular axis, are considered to be the molecules with the greatest incretin effect. Both metabolizable and nonmetabolizable sugars may stimulate GLP-1's secretion (Shima et

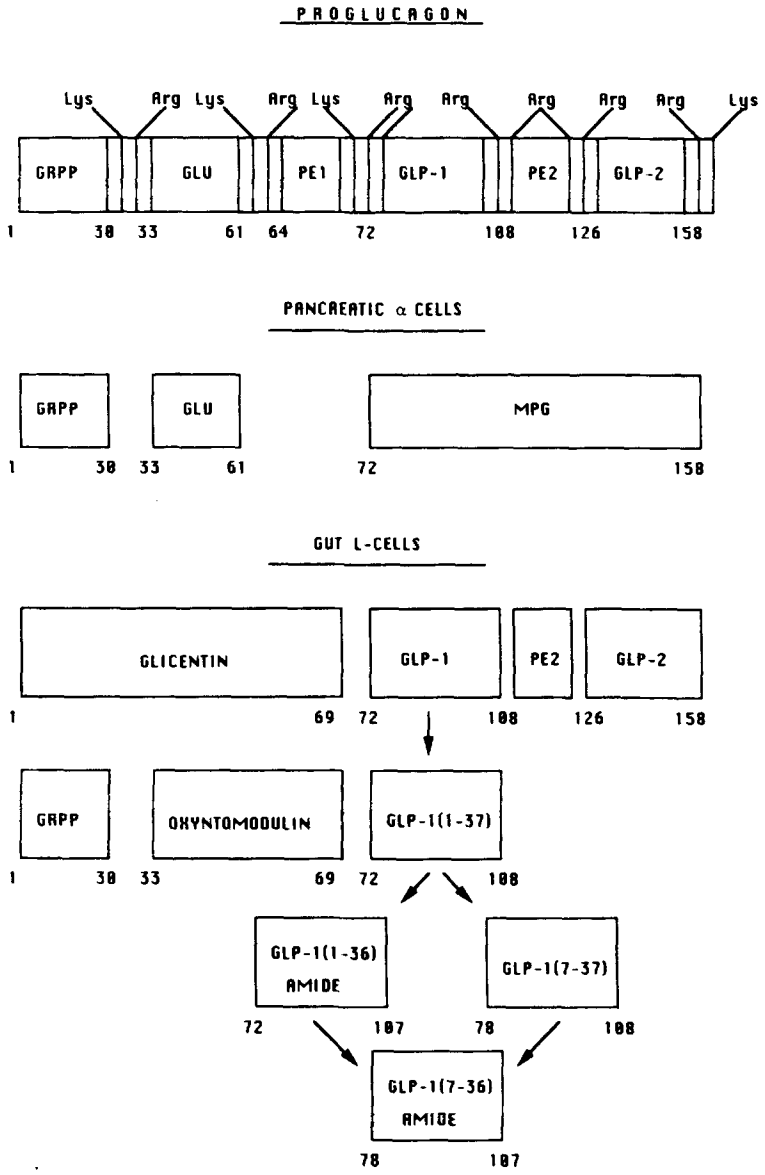

Fig. 1. Schematic presentation of the structure of proglucagon and its post translational processing in pancreas and intestine. GRPP: glicentin-related pancreatic polypeptide. GLU: glucagon. MPGF: major proglucagon fragment. PE: intervening peptide.

al., 1990). Since inhibitors of glucose carriers and of Na^+-K^+- adenosinetriphosphatase activity blocks GLP-1's secretion, it has been suggested that secretion would be associated with glucose transport (Sugiyama et al., 1994). Neuropeptides, such as substance P and gastrin-releasing peptide, and hormones, such as gastric inhibitory peptide (GIP), stimulate GLP-1s' release (Herrmann et al., 1993). GLPs are rapidly removed from the blood circulation, mainly by the kidneys and to a minor extent by the actions of the enzyme dipeptidyl-peptidase IV present in blood plasma. Thus, GLP-1 (7–36) amide has a half-life of 4.5 min,

whereas for GLP-1 (1–37) and GLP-2, this is 8.2 and 8.8 min, respectively (Ruiz-Grande et al., 1990).

Exendin-4 is a peptide isolated from *Helodermatidae* venoms (Eng et al., 1992) that shows 53% structural homology with GLP-1 (7–36) amide. This peptide competes with GLP-1 (7–36) amide at the same binding sites and produces the same chemical mediator and biological effects. Both GLP-1 (7–36) amide and exendin-4 specifically interact with the same receptor on dispersed pancreatic acini, insulinoma-derived cells, and brain and lung membranes. Furthermore, these two peptides increase the production of cyclic AMP by pancreatic acini (Raufman et al., 1992) and insulinoma-derived cells (Göke et al., 1993), and also stimulate glucose-induced insulin secretion in isolated pancreatic islets and proinsulin gene expression at the transcriptional level in mouse insulinoma βTC-1 cells (Göke et al., 1993). They also increase arterial blood pressure and heart rate (Barragán et al., 1994, 1996) in rats. By contrast, exendin (9–39) reduces or inhibits all the above effects of exendin-4 and GLP-1 (7–36) amide. The properties of exendin-4 as an agonist and of exendin (9–39) as a potent antagonist for the GLP-1 receptor open the possibility of using these peptides as tools to define the role of GLP-1 (7–36) amide in different physiological effects. It is interesting that a non mammalian peptide, such as exendin-4, should interact with mammalian target tissues with a more prolonged effect than the endogenous ligand GLP-1 (7–36) amide. The differences in the actions of these two peptides may to owing to a higher affinity of exendin-4 for common receptors (Göke et al., 1993) and/or to a delayed disappearance of exendin-4 from the blood circulation. In accordance with the latter suggestion, GLP-1 (7–36) amide would be a good substrate for the membrane-bound ectopeptidase NEP 24.11 (Hupe-Sodmann et al., 1995), an enzyme that is important for the extracellular processing of neuropeptides. By contrast, exendin-4 is a poor substrate for NEP 24.11, which may explain its longer half-life and may be significant for the use of these peptides as therapeutic agents in diabetes mellitus (Gutniak et al., 1992).

Mechanisms of Actions and Physiological Effects of GLP on Peripheral Tissues

Cloning of the human (Dillon et al., 1993; Thorens et al., 1993) and rat (Thorens, 1992) GLP-1 receptor from pancreatic islets has significantly improved our understanding of the mechanisms of action of this peptide. GLP-1 receptor cDNA encodes a 463 amino acid protein that is identical to the protein from lung, heart, stomach, kidney, and brain. No sequences corresponding to the cloned rat islet GLP-1 receptor are expressed in liver, adipose tissue, or skeletal muscle (Wei and Mojsov, 1995; Bullock et al., 1996). The receptor displays 90% homology in the rat and human. It is a member of the seven-transmembrane family of G-protein-linked receptors, such as receptors for vasoactive intestinal polypeptide (VIP), calcitonin, glucagon, pituitary adenylate cyclase activating peptide (PACAP), secretin, parathyroid hormone (PTH), and growth hormone-releasing hormone (GHRH). Higher sequence homologies are present in the second and sixth membrane-spanning domains, but there is poor sequence conservation in their extracellular domains. The molecular mass of rat islet GLP-1 receptor is different from that of brain and lung, probably because of modifications in postranslational processing, since the sequences of cDNA are identical in all cases. Differences in the molecular mass of the rat GLP-1 receptor from brain as compared with that identified in lung membranes have been investigated by expression of the cDNA of the GLP-1 receptor cloned from pancreatic islets in different cell lines. GLP-1 receptor expressed in CHO and neuroblastoma N_2A cells was examined by SDS-PAGE after chemical crosslinking with ^{125}I-GLP-1 (7–36) amide. As shown in Fig. 2, the GLP-1 receptor was identified as a single ligand binding protein complex. This band

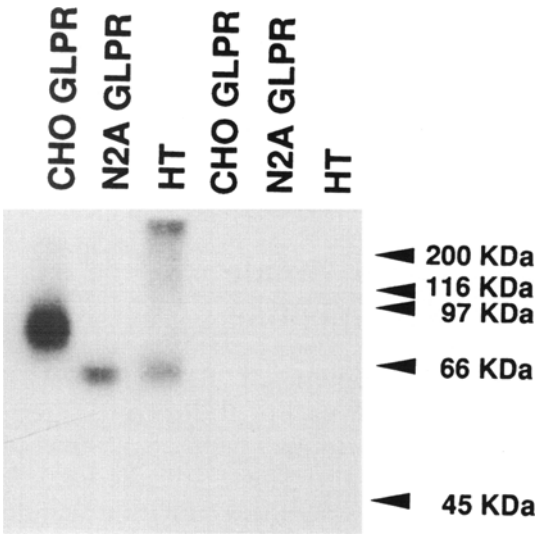

Fig. 2. Crosslinking of ^{125}I-GLP-1 (7–36) amide to hypothalamic membranes and to CHO or N2A cell lines. Membranes from rat hypothalamus (HT) or CHO and N2A cell lines expressing the GLP-1 receptor (CHO-GLPR and N2A GLPR) were incubated with ^{125}I-GLP-1 (7–36) amide in the absence (–) or presence (+) of 500 n*M* unlabeled GLP-1 (7–36) amide (tGLP), followed by treatment with 0.5 m*M* DSS. Crosslinked membranes were solubilized and subjected to SDS-PAGE. Autoradiogram of a representative dried gel is shown. The relative molecular weights of marker proteins are indicated with arrowheads. DSS: dissuccinimidyl suberate.

was not detectable when incubations were carried out in the presence of 500 n*M* unlabeled GLP-1 (7–36) amide. However, the apparent molecular mass of the ligand binding protein was 69 kDa in CHO cells and of 54 kDa in N_2A cells and in hypothalamus. These findings suggest that the differences in molecular mass may be related to a distinctive posttranslational processing of the protein, especially because the nucleotide sequence of the GLP-1 receptor in rat brain is identical to that described from rat pancreatic islets (Alvarez et al., 1996).

Experimental evidence suggests that the GLP-1 receptor in β pancreatic cells and gastric glands is coupled to the adenylate cyclase system and to protein kinase A stimulation (Drucker et al., 1987). Also, the amount of mRNA for the GLP-1 receptor in RINm5F cells is negatively regulated by dexamethasone (Richter et al., 1990) and by agents that modify intracelullar cAMP contents.

Specific high-affinity binding sites for GLP-1 (7–36) amide have been identified in rat insulinoma cells (Göke and Conlon, 1988) pancreatic β-cells, gastric glands (Uttenthal and Blázquez, 1990), and adipocyte (Valverde et al., 1993), lung (Richter et al., 1991), and brain membranes (Shimizu et al., 1987; Kanse et al., 1988; Uttenthal et al., 1992; Calvo et al., 1995a). The entire GLP-1 sequence or GLP-1 (1–37) has low biological activity, and the effects of GLP-2 include the activation of hypothalamic and pituitary adenylate cyclase in rats (Hoosein and Gurd, 1984), and the activation of small bowel epithelial proliferation (Drucker et al., 1996). However the truncated forms of GLP-1 are very active. GLP-1 (7–37) and GLP-1 (7–36) amide are indistinguishable in their ability to stimulate insulin secretion in a glucose-dependent manner in experimental animals and healthy human subjects (Kreymann et al., 1987; Mojsov et al., 1987; Gutniak et al., 1992). These peptides also increase the release of somatostatin and reduce glucagon secretion (Holst and Ørskov, 1994). It has been proposed that the binding of the truncated forms of GLP-1 to their receptors in the pancreatic β-cells increases cAMP levels, which activate protein kinase A, which in turn phosphorylates regulatory sites on ATP-sensitive potassium channels. At the same time, glucose modulates potassium channels via the ATP generated in glycolysis. These two effects favor the closure of ATP-sensitive potassium channels and the depolarization of β-cells, which causes voltage-sensitive calcium channels to open. This then elicits an increase in intracellular calcium levels, which in turn induces the exocytosis of insulin.

GLP-1 (7–36) amide also has significant effects on gastrointestinal motility and secretion. The peptide inhibits gastric acid secretion, gastric emptying, and meal-induced pancreatic exocrine secretion (Schojoldager et al., 1989;

Wettergren et al., 1993; Layer et al., 1996), although the latter phenomena may be secondary to its effects on gastric emptying. These effects seem to be centrally mediated, because they are not found in vagotomized subjects (Ørskov et al., 1995). In addition, intracerebroventricular (icv) administration of GLP-1 (7–36) amide inhibits food intake (Schick et al., 1994; Navarro et al., 1994, 1996; Lambert et al., 1994; Turton et al., 1996; Tang-Christensen et al., 1996), and increases arterial blood pressure and heart rate (data not shown).

High levels of GLP-1 receptors and their mRNA have been found in the lung (Richter et al., 1991; Bullock et al., 1996). It is also know that GLP-1 (7–36) amide binds to receptors on the submucosal glands of the trachea and the smooth muscle of the pulmonary arteries, producing an increase in mucous secretion and pulmonary smooth muscle relaxation (Richter et al., 1993).

The presence of binding sites and the physiological effects of GLP-1 (7–36) amide on skeletal muscle, liver, and adipose tissue are contradictory. Whereas in some reports, a potent glycogenic effect of GLP-1 (7–36) amide has been described in isolated hepatocytes and skeletal muscle (Valverde et al., 1994; Villanueva-Peñacarrillo et al., 1994), in other reports this peptide did not have an effect on cAMP or carbohydrate metabolism, and GLP-1 receptors were not found in rat liver membranes (Ghiglione et al., 1985; Fürnsinn and Ebner, 1995). Since GLP-1 receptor mRNA was not detected by RNase protection, RT-PCR or *in situ* hybridization in skeletal muscle, liver, and adipose tissue, it has been concluded that the pancreatic GLP-1 receptor is not synthesized in these tissues (Wei and Mojsov, 1995; Bullock et al., 1996). A likely explanation for this paradox would be the existence of isotypes of the pancreatic receptor or homologous receptors encoded by different genes.

The truncated forms of GLP-1 have been shown to have antidiabetogenic effects and have been suggested to be useful in the treatment of diabetes mellitus types I and II (Gutniak et al., 1992). In this regard, exendin-4 has a longer functional half-life (Barragán et al., 1996) than GLP-1 (7–36) amide, which may be of potential significance for the use of these peptides as therapeutic agents in diabetes mellitus.

GLP-1 (7–36) Amide as a Neuropeptide

The development of RIAs in the 1970s revealed that several peptides considered gastrointestinal hormones were also found in the mammalian brain. These so-called gut-brain or regulatory peptides include cholecystokinin, enterostatin, bombesin, glucagon, galanin, growth hormone-releasing factor, neuropeptide Y, and others, and are involved in gastrointestinal secretion and motility, and modulate appetite, energy balance, and body weight. Recent findings indicate that GLP-1 (7–36) amide might be one of these regulatory peptides.

Current experimental evidence shows that both GLP-1 (7–36) amide and its own receptors are synthesized in selective areas of the brain, mainly in the hypothalamus and brainstem. These locations suggest that GLP-1 (7–36) amide might act as a neuropeptide involved in autonomic and neuroendocrine activities by regulating several functions, such as food intake, water balance, arterial blood pressure, heart rate, body temperature, and the selective release of neurotransmitters. These effects could be mediated by GLP-1 (7–36) amide of either intestinal or brain origin. GLP-1 (7–36) amide secreted from the intestinal L-cells might enter into the brain by binding to blood –brain barrier-free organs, such as the subfornical organ and the area postrema (Ørskov et al., 1996), or may be transported into the brain through the choroid plexus, which has a high density of GLP-1 receptors (Alvarez et al., 1996). Therefore, GLP-1 (7–36) amide released peripherally may be responsible for some of the central physiological effects of this peptide

in addition to brain GLP-1 (7–36) amide, which may be used for the same or other biological activities in the CNS.

Localization of Glucagon-Like Peptides in the Brain

The proglucagon gene is expressed in the brainstem and hypothalamus of the rat (Drucker and Asa, 1988), and the proglucagon mRNA transcript is identical to that produced in pancreas and intestine, giving rise to glucagon-related peptides in the brain (Shimizu et al., 1987; Jin et al., 1988; Kreymann et al., 1989; Suda et al., 1989). Posttranslational processing of the precursor yields different products in these organs, such products being similar in the rat brain to those found in the intestine (Jin et al., 1988; Kreymann et al., 1989). Although the highest concentrations of GLP-1 (7–36) amide have been detected in the hypothalamus, the mRNA of proglucagon was 100-fold lower in this region than in the brainstem (Drucker and Asa, 1988). These findings have been interpreted to suggest that proglucagon mRNA is less abundant, but is translated or stored more efficiently in the hypothalamus than in the brainstem (Drucker and Asa, 1988). Furthermore, the possibility exists that glucagon-like peptides might be synthesized in the brainstem and transported along brainstem afferents to nuclei in the hypothalamus. This suggests that the peptide would actually be synthesized in the brain and that it is not a consequence of being taken up from the blood circulation. GLP-1 (7–36) amide content in the brain has been determined by RIA, with the highest concentration being found in hypothalamus (Table 1), followed at a lower level by the midbrain, and pons and medulla oblongata (Kreymann et al., 1989). Cell bodies with immunoreactivity for GLP-1 have been identified in the nucleus tractus solitarius and in the ventral and dorsal part of the medullary reticular nucleus. Also, GLP-1-immunoreactive nerve fibers have been found in several brain regions, the highest contents being found in the hypothalamus.

Table 1
Regional Distribution of GLP-1 (7–36) Amide in Rat Brain[a]

Region	Content of GLP-1 (7–36) Amide
Hypothalamus	++++
Midbrain	++
Pons	+
Medula oblongata	++
Cerebral cortex	+
Cerebellum	+
Pituitary gland	+

[a]The content of GLP-1 (7–36) amide has been rated on a scale of + (low) to ++++ (high).

Distribution of GLP-1 Receptors in the Brain

Several experimental approaches have been used to identify GLP-1 receptors in the brain. These methods include measurement of the stimulation of cAMP production by hypothalamic homogenates (Hoosein and Gurd, 1984, Shimizu et al., 1987) displaceable binding by crude membrane or synaptosomal fractions (Shimizu et al., 1987; Kanse et al., 1988; Calvo et al., 1995a), crosslinking experiments (Calvo et al., 1995a), and quantitative autoradiography of brain sections (Utthental et al., 1992; Göke et al., 1995a, b). In addition, the gene expression of the GLP-1 receptor has been studied by biochemical and *in situ* hybridization histochemical methods (Alvarez et al., 1996; Navarro et al., 1996; Bullock et al., 1996). Specific binding sites for GLP-1 (7–36) amide have been detected in several rat brain areas (Table 2), with the highest values being found in hypothalamus, thalamus, brainstem, lateral septum, the subfornical organ, and area postrema, whereas other regions, such as the cerebral cortex, caudate-putamen, and cerebellum, exhibit low to negligible values (Uttenthal et al., 1992; Campos et al., 1994; Calvo et al.,

Table 2
Regional Distribution of GLP-1 Receptors in the Rat Brain[a]

Region	Content of GLP-1 Receptors
Hypothalamus	++++
Brainstem	+++
Cerebral cortex	+
Caudate putamen	++
Cerebellum	+
Nuclei	
Arcuate	++++
Ventromedial hypothalamic	++++
Dorsomedial hypothalamic	+++
Posterior paraventricular thalamic	++
Anterior paraventricular thalamic	+
Nucleus of the solitary tract	+++
Pituitary gland	++

[a] The content of GLP-1 receptors has been rated on a scale of + (low) to +++ (high).

1995a; Göke et al., 1995a). The highest densities of GLP-1 receptors in brain nuclei (Table 2) are found in the arcuate, ventromedial and dorsomedial hypothalamic nuclei of the solitary tract, the posterodorsal tegmental nucleus, and the interpeduncular nucleus (Uttenthal et al., 1992; Calvo et al., 1995a; Göke et al., 1995a). The interaction of GLP-1 (7–36) amide with brain homogenates meets the generally accepted criteria for peptide hormone – receptor interaction: time, temperature and protein content dependency, high affinity, saturability, and specificity. Identification of brain GLP-1 (7–36) amide binding proteins by covalent crosslinking to radiolabeled GLP-1 (7–36) amide and then analyzing the resulting complexes on SDS-PAGE, affords an M_r of 56-kDa protein specifically labeled in brain homogenates (Calvo et al., 1995a), whereas in lung membranes, the specifically labeled band was an M_r 52.5-kDa protein. The electrophoretic mobility of these GLP-1 (7–36) amide-binding proteins did not change under reducing conditions, suggesting that they do not contain any interchain disulfide bonds within their structure and that they are not part of a larger disulfide-linked protein. Covalent M_r 56-kDa GLP-1 (7–36) amide binding protein complexes solubilized by Triton X-100 have been adsorbed onto wheat germ agglutinin. The results suggest that the GLP-1 receptor in rat brain is a glycoprotein with a single binding subunit that has higher molecular mass, but binding features and ligand specificity similar to those of its peripheral tissue counterparts (Calvo et al., 1995a).

GLP-1 receptor cDNA from human (Wei and Mojsov, 1995) and rat (Alvarez et al., 1996) brains has been cloned and sequenced, and the deduced amino acid sequences are the same as the sequence found in pancreatic islets. *In situ* hybridization histochemical studies have revealed specific labelling of mRNA of GLP-1 receptors in both neurons and glia of the thalamus, hypothalamus, hippocampus, primary olfactory cortex, choroid plexus, and pituitary gland (Alvarez et al., 1996; Navarro et al., 1996). These findings indicate that GLP-1 receptors are indeed synthesized in rat brain. The colocalization of GLP-1 receptors, glucokinase and GLUT-2 in the same areas (Navarro et al., 1996) supports the idea that these cells play an important role in glucose sensing in the brain. It seems noteworthy that the nucleus of the solitary tract is the site of both GLP-1 (7–36) amide-synthesizing neurons and high GLP-1 receptor density. This suggests that GLP-1 (7–36) amide has a local as well as a more remote signaling role in this nucleus, which is involved in the central control of cardiovascular function and also acts as a sensory nucleus for taste. In addition, high concentrations of GLP-1 (7–36) amide and its own receptors are present in the same hypothalamic nuclei, especially in those involved in the control of food intake. Close neuroanatomical connections exist between these hypothalamic areas and those of the subfornical organ and the area postrema, which have GLP-1 receptors accessible to peripherally administered GLP-1 (7–36) amide.

Other localizations of GLP-1 receptors may provide additional information about the actions of this peptide. Specific high-affinity

binding sites for GLP-1 (7–36) amide have been determined in the pituitary gland (Kanse et al., 1988; Calvo et al., 1995a), and this peptide stimulated basal TSH release from dispersed anterior pituitary cells in a concentration-dependent manner (Beak et al., 1996). GLP-1 receptors also have been characterized in the posterior lobe of the rat pituitary (Göke et al., 1995b), and icv, administration of GLP-1 (7–36) amide significantly increases vasopressin secretion (Tang-Christensen et al., 1996).

Actions of GLP-1 (7–36) Amide on the Brain

Although earlier studies indicated that GLP-1 (7–36) amide is a potent insulin secretagog, later it was found that several biological effects of this peptide are mediated via the brain, rather than in peripheral tissues. Such actions include the effects of GLP-1 (7–36) amide on the release of neurotransmitters from selective brain nuclei, the inhibition of gastric acid secretion and motility, food and water intake, thermoregulation, and arterial blood pressure (unpublished results).

These effects, together with the presence of GLP-1 (7–36) amide in the synaptosome fraction, its calcium-dependent release by potassium stimulation (Kreymann et al., 1989), and the colocalization of this peptide and its own receptors in the same brain regions, suggest that GLP-1 (7–36) amide may act as a neurotransmitter. However, the application of GLP-1 (7–36) amide (A. Colinos, personal communication) for 15 min had no effect on field excitatory postsynaptic potentials on the amplitude of the presynaptic fiber volley recorded in the stratum radiatum in the CA1 region of hippocampal slices. These results indicate that GLP-1 (7–36) amide has no effect on presynaptic axonal excitability or on synaptic efficiency in the hippocampus, although they do not rule out the possibility that such effects might be positive in other brain regions. Further ultrastructural and electrophysiological studies are needed to define GLP-1 (7–36) amide as a neurotransmitter or as a neuromodulator. However, we now have sufficient evidence to state that it functions as a neuropeptide.

Release of Neurotransmitters by Perfusion of GLP-1 (7–36) Amide in Brain Areas of the Conscious Rat

The effects of GLP-1 (7–36) amide on the release of amino acid neurotransmitters in the basal ganglia of conscious rats have been studied after perfusion of the compound through a concentric "push-pull" cannula system with artificial cerebrospinal fluid. Perfusion with GLP-1 (7–36) amide produced an immediate increase in extracellular levels of glutamine and glutamic acid, but the peptide had no effect on the levels of aspartic acid, glycine, or serine (Mora et al., 1992). The action elicited by GLP-1 (7–36) amide on the release of glutamine and glutamic acid could be interpreted as a direct effect of the peptide on astrocytes and glutamatergic neurons. It is known that glutamine is almost totally synthesized in astrocytes (Norenberg and Martínez-Hernández, 1979) and that these cells express a wide variety of neurotransmitter receptors (Murphy and Pearce, 1987). Therefore, the possibility exists that GLP-1 (7–36) amide might directly stimulate glial cells through GLP-1 receptors. Moreover, it is possible that the release of glutamic acid could result from the synthesis and release of glutamine by astrocytes, since glutamatergic neurons require active reuptake of glutamine to synthesize glutamic acid (Hertz and Schousboe, 1986).

By means of an experimental microdialysis approach, the effects of GLP-1 (7–36) amide on the release of excitatory amino acid neurotransmitters by the ventromedial hypothalamus have been studied (Calvo et al., 1995b). GLP-1 (7–36) amide produced an immediate increase in the extracellular concentrations of aspartic acid and glutamine, but the concentrations of glutamic acid, alanine, threonine, and tyrosine were unaffected. Interestingly, two brain areas, i.e., the ventromedial hypothalamus and the basal ganglia, display a distinc-

tive pattern of neurotransmitter release into the extracellular space after perfusion with GLP-1 (7–36) amide. Thus, in the ventromedial hypothalamus, this peptide increases extracellular concentrations of aspartate and glutamine, whereas in the basal ganglia, it produces a selective release of glutamine and glutamic acid (Mora et al., 1992). These findings could be related to the effects of the peptide on different neurochemical substrates in these two brain areas. It is interesting too that another regulatory peptide, namely cholecystokinin, also produces a release of aspartate by the ventromedial hypothalamus (Barners et al., 1991). Since this brain area is involved in the control of food intake and since both cholecystokinin and GLP-1 (7–36) amide reduce food ingestion, it could be suggested that these peptides might participate in the central control of feeding behavior through the release of aspartate (Calvo et al., 1995b).

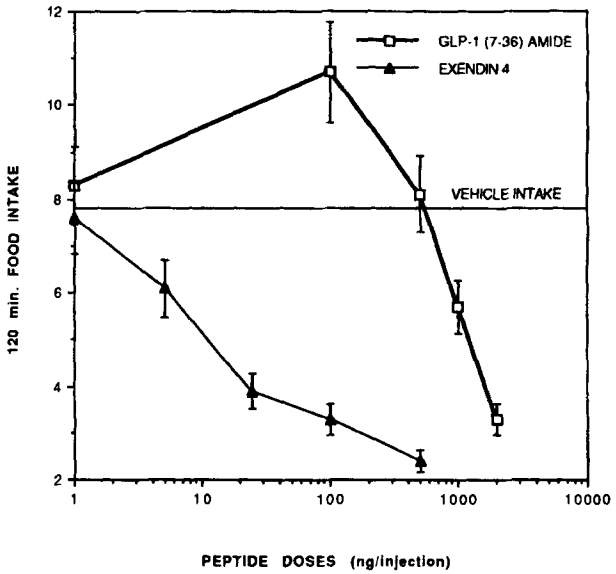

Fig. 3. Effects of acute icv administration of either GLP-1 (7–36) amide or exendin-4 on cummulated food intake in 24-h food-deprived rats. Data are means ± SEM.

Effect of GLP-1 (7–36) Amide on Food and Drink Intake

Acute or subchronic ip administration of GLP-1 (7–36) amide does not modify food and water intake. In contrast, icv administration of GLP-1 (7–36) amide produces a biphasic effect on food intake characterized by an increase in the amount of food intake after acute icv delivery of 100 ng of the peptide. A marked reduction (Fig. 3) in food ingestion (Schick et al., 1994; Navarro et al., 1994, 1996; Lambert et al., 1994; Turton et al., 1996; Tang-Christensen et al., 1996) has been observed at 1000 and 2000 ng, or even greater doses of the peptide, which also produce a significant decrease in water intake. When given by icv administration, exendin-4 proved to be a potent agonist of GLP-1 (7–36) amide in that it decreased both food and water intake in a dose-dependent manner (Fig. 4). Pretreatment by icv administration of exendin (9–39) reversed the inhibitory effects of GLP-1 (7–36) amide or of exendin-4. These findings suggest that GLP-1 (7–36) amide may modulate both food and drink intake in the rat through a central mechanism (Navarro et al., 1996; Tang-Christensen et al., 1996). Recently, it has been reported that this peptide has the same effect in humans when administered peripherally at high doses (Gutzwiller et al., 1997).

Decreases in water intake (Fig. 4) could simply be associated with the effects of food intake However, several observations suggest a possible action of GLP-1 (7–36) amide on thirst-regulatory mechanisms, such as the fact that the effects of water intake do not always parallel food intake measurements. Furthermore, GLP-1 receptor mRNA has been located in brain areas related to the control of thirst, such as the preoptic area, glial cells lining the third ventricle, and, especially, the neurons of the paraventricular nucleus (Alvarez et al., 1996), which is a key station for water balance regulation, through the antidiuretic effects of vasopressin released by its projection to the neurohypophysis. In addition, icv administration of GLP-1 (7–36) amide significantly increases the circu-

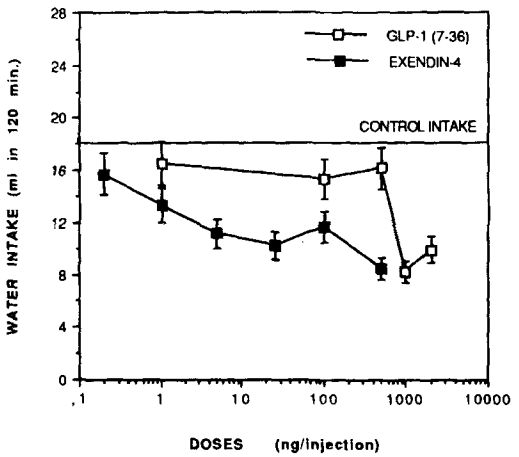

Fig. 4. Effects of acute icv administration of either GLP-1 (7–36) amide or exendin-4 on water intake in rats. Water intake was measured 120 min after peptide delivery. Data are means ± SEM.

lating levels of vasopressin (Tang-Christensen et al., 1996), and colocalization of the mRNAs of the GLP-1 receptor and vasopressin has been found in the neurons of the paraventricular nucleus (Zullo et al., in press).

The use of *in situ* hybridization has allowed the identification of the mRNA of the GLP-1 receptor in limited populations of brain cells, including the ependymal cells lining the third ventricle (Fig. 5) and cells of the lateral and ventromedial hypothalamic nuclei (Alvarez et al., 1996; Navarro et al., 1996). These areas are considered to be glucose-sensitive or glucose-responsive (Oomura et al., 1992; Treherme and Ashford, 1992), and have been reported to mediate feeding behavior and to participate through hormonal and autonomic mechanisms in the modulation of pancreatic islet function. In the medial and lateral hypothalamus, the presence of glucokinase, and GLUT-2 (Jetton et al., 1994; Alvarez et al., 1996; Navarro et al., 1996) and GLP-1 receptors (Alvarez et al., 1996; Navarro et al., 1996) has been reported. GLUT-2 and glucokinase are proteins involved in the high K_m glucose transporter and in the high K_m phosphorylation of glucose, respectively, which play important roles in the multistep process of glucose sensing. The pancreatic β-cell is the only cell type for which a biochemical mechanism for glucose sensing has been elucidated. In considering this model of glucose sensing, we suggest that increased glycemia after meals may be recognized in the hypothalamus by the aforementioned cells owing to the presence of GLUT-2 and glucokinase. Furthermore, the metabolites arising during glucose oxidation in these cells may be involved in the transduction of the signals needed to produce a state of fullness. Presently, it is known that in addition to pancreatic β-cells, at least the ependymal cells in the third ventricle have GLP-1 receptors, glucokinase, GLUT-2 (Alvarez et al., 1996; Navarro et al., 1996), and ATP-sensitive K^+ channels (Treherme and Ashford, 1992), and display glucose-dependent changes in their membrane potential.

Actions of GLP-1 (7–36) Amide on Gastric Acid Secretion and Motility and on Thermoregulation

GLP-1 (7–36) amide inhibits gastric acid secretion, gastric emptying, and pancreatic enzyme secretion (Schjoldager et al., 1989; Wettergren et al., 1993; Layer et al., 1996). These effects of GLP-1 (7–36) amide are not found in vagotomized subjects, suggesting a centrally mediated effect (Ørskov et al., 1995).

A role for GLP-1 in temperature regulation also has been reported (O'Shea et al., 1996). Thus, administration of high doses of GLP-1 icv caused a significant reduction in body temperature over the subsequent 2 h, which was blocked by prior icv administration of exendin (9–39). This effect may be induce peripherally. Thus, the ip injection of 300 ng of GLP-1 reduced temperature over 2 h, but had no effect on food intake. Accordingly, it has been proposed that, given the effect on temperature but not food intake of ip GLP-1, the mechanism regulating the effect of GLP-1 on ther-

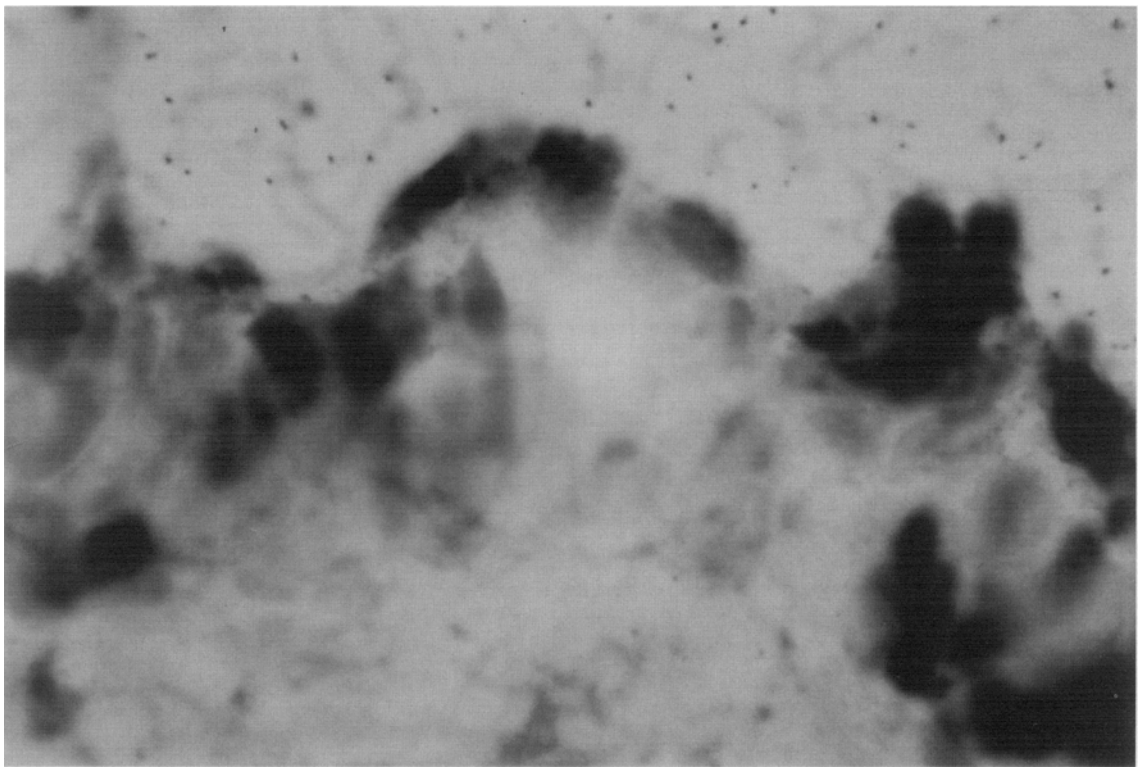

Fig. 5. *In situ* hybridization histochemistry of GLP-1 receptor mRNA in the wall of the third ventricle of the rat. Brown reaction product indicates labeling of GLP-1 receptor mRNA (500×).

moregulation may be different from that regulating food intake.

Role of Central GLP-1 (7–36) Amide on Behavior

Recent work suggests additional effects of centrally delivered GLP-1 (7–36) amide, in addition to its actions on feeding and drinking behavior. In this sense, it has been reported that central infusion of this peptide results in the establishment of taste aversion (Thiele et al., 1997; Van Dijk et al., 1997). Although this might account for the satiety-inducing actions of this peptide, this effect appeared after previous exposure to central administration of higher doses of GLP-1 (3 μg) and was not found by another research group (Tang-Christensen et al., 1996) using lower doses (1 μg), which do decrease food intake. Moreover, the presence of GLP-1 receptors in brainstem areas related to gustatory processes, such as the nucleus of the solitary tract (Alvarez et al., 1996), may account for the conditioned rejection of a tasteful solution (saccharin). Additionally, it has recently been reported that central administration of GLP-1 results in alterations in social interaction behaviors in rats (Panksepp et al., 1996), reflecting increased emotionality as a result of peptide infusion. These data are consistent with the described activation of limbic areas, as mapped using c-Fos expression: central administration of GLP-1 increased Fos expression in the central nucleus of the amygdala, the paraventricular nucleus of the hypothalamus, the nucleus of the solitary tract, and the area postrema (Turton et al., 1996; Van Dijk et

al., 1997). Moreover, central administration of GLP-1 (10 μg) results in activation of the hypothalamus-pituitary-adrenal axis, giving rise to an increase in corticosterone levels 30 min after the injection (Wang et al., 1996). These aversive effects of GLP-1 might affect food intake. However, the wide distribution of GLP-1 receptors in limbic areas, as well as in hypothalamic and brainstem nuclei (Shimizu et al., 1987; Kanse et al., 1988; Uttenthal et al., 1992; Calvo et al., 1995; Alvarez et al., 1996; Navarro et al., 1996), precludes a clear-cut analysis of these effects, observed after intraventricular administration of high doses of the peptide. Future research is needed to clarify the contribution of these discrete brain regions to the effects observed after central administration of GLP-1 receptor agonists.

Effects of Glucagon-Like Peptides on Arterial Blood Pressure and Heart Rate

In addition to the pancreatic and extrapancreatic actions of glucagon-like peptides, they also have some cardiovascular effects. Glucagon has positive and chronotropic effects; it affects regional blood circulation and also produces a slight, but significant increase in arterial blood pressure (Parmley et al., 1968). GLP-2 has no effect, and GLP-1 (1–37) produces a moderate increase in arterial blood pressure, whereas GLP-1 (7–36) amide induces a dose-dependent increase in systolic and diastolic arterial blood pressure and heart rate (Barragán et al., 1994). The action of GLP-1 (7–36) amide on these parameters seems to be mediated through its own receptors, since exendin-4 acts as an agonist and exendin (9–39) blocks the effects of this peptide on arterial blood pressure and heart rate (Barragán et al., 1996).

Since both GLP-1 (7–36) amide and its own receptors have been found in significant amounts in the nucleus tractus solitarius (Shimizu et al., 1987; Kanse et al., 1988; Jin et al., 1988; Kreymann et al., 1989; Suda et al., 1989; Uttenthal et al., 1992; Calvo et al., 1995a; Göke et al., 1995a), which is involved in the central control of cardiovascular function (Cechetto, 1987), the possibilities exist that exogenously administered GLP-1 (7–36) amide or this peptide released from brain areas might alter cardiovascular parameters through a central mechanism. Thus, the peptide might enter into the brain by binding to blood–brain barrier-free organs, such as the area postrema and the subfornical organ (Ørskov et al., 1996), it may be transported to the brain by the choroid plexus, which has a high density of GLP-1 receptors (Alvarez et al., 1996), or the peptide of brain origin may be released from locations, such as the nucleus of the tractus solitarius. Accordingly, icv administration of either GLP-1 (7–36) amide produces a dose-dependent increase in systolic and diastolic blood pressure and in heart rate (unpublished results). These findings are reinforced by the facts that the stimulating effects of GLP-1 (7–36) amide on cardiovascular parameters after its iv administration are blocked by icv injection of exendin (9–39) and because bilateral vagotomy abolishes the effect of the icv administration of GLP-1 (7–36) amide on arterial blood pressure. These results indicate that GLP-1 (7–36) amide increases arterial blood pressure and heart rate in the rat through a central mechanism that is transmitted to the periphery through the vagus nerve.

References

Alvarez E., Roncero I., Chowen J. A., Thorens B., and Blázquez E. (1996) Expression of the glucagon-like receptor gene in rat brain. *J. Neurochem.* **66,** 920–927.

Banting F. G. and Best C. H. (1922) The internal secretion of the pancreas. *J. Lab. Clin. Med.* **7,** 251–266.

Barners S., Whistler H. L., Hughes J., Woodruff G. N., and Hunter J. C. (1991) Effect of cholecystokinin octapeptide on endogenous amino acid release from the rat ventromedial nucleus of the hypothalamus and striatum. *J. Neurochem.* **56,** 1409–1416.

Barragán J. M., Rodriguez R., and Blázquez E. (1994) Changes in arterial blood pressure and heart rate induced by glucagon-like peptide-1-(7–36) amide in rats. *Am. J. Physiol.* **266,** E459–E466.

Barragán J. M., Rodriguez R., Eng J., and Blázquez E. (1996) Interactions of exendin-(9–39) with the effects of glucagon-like peptide-1-(7–36) amide and of exendin-4 on arterial blood pressure and heart rate in rats. *Regul. Pept.* **67,** 63–68.

Beak S. A., Small C. J., Ilovaiskaia I., Hurley J. C., Ghatei M. A., Bloom S. R., and Smith D. M. (1996) Glucagon-like peptide-1 (GLP-1) releases thyrotropin (TSH): characterization of binding sites for GLP-1 on α-TSH cells. *Endocrinology* **137,** 4130–4138.

Bell G. I., Santerre R. F., and Mullenbach G. T. (1983) Hamster preproglucagon contains the sequence of glucagon and two related peptides. *Nature* **302,** 716–718.

Bullock B. P., Scottheller R., and Habener J. F. (1996) Tissue distribution of messenger ribonucleic acid encoding the rat glucagon-like peptide-1 receptor. *Endocrinology* **137,** 2968–2978.

Calvo J. C., Yusta B., Mora F., and Blázquez E. (1995a) Structural characterization by affinity cross-linking of glucagon-like peptide-1 (7–36) amide receptor in rat brain. *J. Neurochem.* **64,** 299–306.

Calvo J. C., Gisolfi C. V., Blázquez E., and Mora F. (1995b) Glucagon-like peptide-1 (7–36) amide induces the release of aspartic acid and glutamine by the ventromedial hypothalamus of the conscious rat. *Brain Res. Bull.* **38,** 435–439.

Campos R. V., Lee Y. C., and Drucker D. J. (1994) Divergent tissue-specific and developmental expression of receptors for glucagon and glucagon-like peptide-1 in the mouse. *Endocrinology* **134,** 2156–2164.

Cechetto D. F. (1987) Central representation of visceral function. *Fed. Proc.* **46,** 17–23.

Dillon J. S., Tanizawa Y., Wheeler M. B., Leng X. H., Ligon B. B., Rabin D. H., et al. (1993) Cloning and functional expression of the human glucagon-like peptide-1 (GLP-1) receptor. *Endocrinology* **133,** 1907–1910.

Drucker D. J. and Asa S. (1988) Glucagon gene expression in vertebrate brain. *J. Biol. Chem.* **263,** 13,475–13,478.

Drucker D. J., Philippe J., Mojsov S., Chick W. L., and Habener J. F. (1987) Glucagon-like peptide I stimulates insulin gene expression and increases cyclic AMP levels in a rat islet cell line. *Proc. Natl. Acad. Sci. USA* **84,** 3434–3438.

Drucker D. J., Ehrlich P., Asa S. L., and Brubaker P. (1996) Induction of intestinal proliferation by glucagon-like peptide-2. *Proc. Natl. Acad. Sci. USA* **93,** 7911–7916.

Eng J., Kleiman W. A., Singh L., Singh G., and Raufman J. P. (1992) Isolation and characterization of exendin-4 and exendin-3 analogues from Heloderma suspectum venom. Further evidence for an exendin receptor on dispersed acini from guinea pig pancreas. *J. Biol. Chem.* **267,** 7402–7405.

Fürnsinn C. and Ebner K. W.W (1995) Failure of GLP-1 (7–36) amide to affect glycogenesis in rat skeletal muscle. *Diabetologia* **38,** 864–867.

Ghiglione M., Blázquez E., Uttenthal L. O., de Diego J. G., Alvarez E., George S. K., et al., (1985) Glucagon-like peptide-1 does not have a role in hepatic carbohydrate metabolism. *Diabetologia* **28,** 920–921.

Göke R. and Conlon J. M. (1988) Receptors for glucagon-like peptide-1 (7–36) amide on rat insulinoma-derived cells. *J. Endocrinol.* **116,** 357–362.

Göke R., Fehmann H. C., Linn T., Schmidt H., Krause M., Eng J., et al., (1993) Exendin-4 is a high potency agonist and truncated exendin-9 (9–39) amide an antagonist at the glucagon-like peptide-1-(7–36) amide receptor of insulin-secreting β-cells. *J. Biol. Chem.* **268,** 19, 650–19,655.

Göke R., Larsen P. J., Mikkelsen J. C., and Sheikb S. P. (1995a) Distribution of GLP-1 binding sites in the rat brain: evidence that exendin-4 is a ligand of brain GLP-1 binding sites. *Eur. J. Neurosc* **7,** 2294–2300.

Göke R., Larsen P. J., Mikkelsen J. C., and Sheikh S. P. (1995b) Identification of specific binding sites for glucagon-like peptide-1 on the posterior lobe of the rat pituitary. *Neuroendocrinology* **62,** 130–134.

Gutniak M., Orskov C., Holst J. J., Ahrén B., and Efendic S. (1992) Antidiabetogenic effect of glucagon-like peptide-1 (7–36) amide in normal subjects and patients with diabetes mellitus. *N. Engl. J. Med.* **326,** 1316–1322.

Gutzwiller J. P., Göke B., Drewe J., Ketterer S., Handschin D., Hildebrand B., et al. (1997) Glucagon-like peptide-1 is a physiologic regulator of food intake in humans, in *Invited Workshop: Glucagon-Like Peptide-1 (GLP-1)* 7th Annual Meeting of European Neuropeptide Club. May 21–24, 1997, Marburg, Germany.

Heinrich G., Gross P., and Habener J. F. (1984) Glucagon gene sequence: four of six exons encode separate functional domains of rat preproglucagon. *J. Biol Chem.* **259,** 14082–14087.

Herrmann C., Vöge A., and Göke B. (1993) Regulation of glucagon-like peptide (GLP-1) release from the isolated perfused rat ileum by nutrients, peptides and neuromediators. *Digestion* **54,** 367.

Hertz L. and Schousboe A. (1986) Role of astrocytes in compartimentation of amino acids and energy metabolism, in: *Biochemistry, Physiology and Pharmacology of Astrocytes* (Fedoroff S. and Vernadakis A., eds), Academic, New York, p. 179.

Holst J. J., and Ørskov C. (1994) Glucagon and other proglucagon-derived peptides, in *Gut Peptides: Biochemistry and Physiology* (Walsh J. H. and Dockray G. J., eds), Raven, New York, p. 305.

Hoosein N. M. and Gurd R. S. (1984) Human glucagon-like peptides 1 and 2 activate rat brain adenylate cyclase. *FEBS Lett.* **178,** 83–86.

Hupe-Sodmann K., Mc Gregor G. P., Bridenbaug R., Göke R., Göke B., Thole H., et al. (1995) Characterisation of the processing by human neutral endopeptidase 24.11 of GLP-1 (7–36) amide and comparison of the substrate specificity of the enzyme for other glucagon-like peptides. *Regul. Pept.* **58,** 149–156.

Jetton T. L., Liang Y., Petepher C. C., Zimmerman E. C., Cox F. G., Horvath K., et al. (1994) Analysis of upstream glucokinase promoter activity in transgenic mice and identification of glucokinase in rare neuroendocrine cells in the brain and gut. *J. Biol. Chem.* **269,** 3641–3654.

Jin S. L. C., Han V. K. M., Simmons J. E., Towle A. C., Lander J. M., and Lund P. K. (1988) Distribution of glucagon-like peptide-1 (GLP-1), glucagon and glicentin in the rat brain: an immunocytochemical study. *J. Comp. Neurol.* **271,** 519–532.

Kanse S. M., Kreymann B., Ghatei M. A., and Bloom S. R. (1988) Identification and characterization of glucagon-like peptide-1 (7–36) amide binding sites in the rat brain and lung. *FEBS Lett.* **241,** 209–212.

Kimball C. P. and Murlin J. R. (1923) Aqueous extracts of pancreas. III. Some precipitation reactions of insulin. *J. Biol. Chem.* **58,** 337–346.

Kreymann B., Williams G., Ghatei M. A., and Bloom S. R. (1987) Glucagon-like peptide-1 (7–36): a physiological incretin in man. *Lancet* **2,** 1300–1304.

Kreymann B., Ghatei M. A., Burnet P., Williams G., Kanse S., Diani A. R., et al. (1989) Characterization of glucagon-like peptide-1 (7–36) amide in the hypothalamus. *Brain Res.* **502,** 325–331.

Lambert P. C., Wilding J. P. H., Ghatei M. A., and Bloom S. R. (1994) A role for GLP-1 (7–36) NH_2 in the central control of feeding behavior. *Digestion* **54,** 360–361.

Layer P., Franke A., Holst J. J., Grandt D., and Goebell H. (1996) Glucagon-like peptide-1 (GLP-1) inhibits pancreatic enzyme secretion in humans. Gastroenterology **110,** F1409.

Mojsov S., Heinrich G., Wilson I. B., Ravazzola M., Orci L., and Habener J. F. (1986) Preproglucagon gene expression in pancreas and intestine diversifies at the level of post-transcriptional processing. *J. Biol. Chem.* **261,** 11880–11889.

Mojsov S., Weir G. C., and Habener J. F. (1987) Insulinotropin: glucagon-like peptide-1 (7–37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. *J. Clin. Invest.* **79,** 616–619.

Mora F., Expósito I., Sanz B., and Blázquez E. (1992) Selective release of glutamine and glutamic acid produced by perfusion of GLP-1 (7–36) amide in the basal ganglia of the conscious rat. *Brain Res. Bull.* **29,** 359–361.

Murphy S. and Pearce B. (1987) Functional receptors for neurotransmitters on astroglial cells. *Neuroscience* **22,** 381–394.

Navarro M., Rodriguez de Fonseca F., Zueco J. A., Gómez R., and Blázquez E. (1994) Changes in food intake induced by GLP-1 (7–36) amide in the rat Abstract in *15th International Diabetes Federation Congress,* Kobe, Japan.

Navarro M., Rodriguez de Fonseca F., Alvarez E., Chowen J. A., Zueco J. A., Gómez R., et al. (1996) Colocalization of glucagon-like peptide-1 (GLP-1) receptors, glucose transporter GLUT-2, and glucokinase mRNAs in rat hypothalamic cells: Evidence for a role of GLP-1 receptor agonists as an inhibitory signal for food and water intake. *J. Neurochem.* **67,** 1982–1991.

Norenberg M. C. and Martínez-Hernández A. (1979) Fine structural localization of glutamine-synthethase in astrocytes of rat brain. *Brain Res.* **161,** 303–310.

Oomura Y., Sasaki K., Suzuki K., Muto T., Li A. J., Ogita Z., et al. (1992) A new brain glucosensor and its physiological significance. *Am. J. Clin. Nutr.* **55,** 2785–2825.

Ørskov C., Rabenhoj L., Kofod H., Wettergren A., and Holst J. J. (1994) Production and secretion of amidated and glycine-extended glucagon-like peptide-1 (GLP-1) in man. *Diabetes* **43,** 535–539.

Ørskov C., Wettergren A., Poulsen S. C. S., and Holst J. J. (1995) Is the effect of glucagon-like peptide-1 on gastric emptying centrally mediated. *Diabetologia* **38 (Suppl. 1),** A39 (Abstract).

Ørskov C., Poulsen S. S., Moller M., and Holst J. J. (1996) Glucagon-like peptide I receptors in the subfornical organ and the area postrema are accesible to circulating glucagon-like peptide I. *Diabetes* **45,** 832–835.

O'Shea D., Gunn I., Chen X., Bloom S., and Herbert J. (1996) A role for central glucagon-like peptide-1 in temperature regulation. *Neuroreport* **7,** 830–832.

Panksepp J., Bekkedal M. Y. U., and Walter M. (1996) Potent suppresive effects of the putative agent GLP-1 on social-emotional behaviors. *Soc. Neurosci. Abstrat* **22,** p 16.

Parmley W. W., Glick G., and Sonnenblick P. (1968) Cardiovascular effects of glucagon in man. *N. Engl. J. Med.* **279,** 12–17.

Raufman J. P., Singh L., and Eng J. (1992) Truncated glucagon like peptide-1 interacts with exendin receptors on dispersed acini from guinea pig pancreas. Identification of a mammalian analogue of the reptilian peptide exendin-4. *J. Biol Chem.* **267,** 21,432–21,437.

Richter G., Göke R., Göke B., and Arnold R. (1990) Dexamethasone pretreatment of rat insulinoma cells decreases binding of glucagon-like peptide-1 (7–36) amide. *J. Endocrinol.* **126,** 445–450.

Richter G., Göke G., Göke B., Schmidt H., and Arnold R. (1991) Characterization of glucagon-like peptide-1 (7–36) amide receptors of rat lung membranes by covalent cross-linking. *FEBS Lett.* **280,** 247–250.

Richter G., Feddersen O., Wagner U., Barth P., Göke R., and Göke B. (1993) GLP-1 stimulates secretion of macromolecules from airways and relaxes pulmonary artery. *Am. J. Physiol.* **265,** L374–L381.

Ruiz-Grande, C., Pintado J., Alarcón C., Castilla C., and Valverde I. (1990) Renal catabolism of human glucagon-like peptides 1 and 2. *Can. J. Physiol. Pharmacol.* **68,** 1568–1573.

Schjoldager B. T. G., Mortensen P. E., Christiansen J., Ørskov C., and Holst J. J. (1989) GLP-1 (glucagon-like peptide-1) and truncated GLP-1, fragments of human proglucagon, inhibit gastric acid secretion in man. *Dig. Dis. Sci.* **35,** 703–708.

Shick R. R., Worm Walde T., Zimmerman J. P., Schusdziarra V., and Classen M. (1994) Glucagon-like peptide 1: a novel brain peptide involved in feeding regulation in: *Obesity in Europe* (Ditschuneit H., Gries F. A., Hanner H., Schusdziarra V., and Wechsler J. G., eds.), Libbey, London, 363–367.

Shima K., Suda T., Nishimoto K., and Yoshimoto S. (1990) Relationship between molecular structures of sugars and their ability to stimulate the release of glucagon-like peptide-1 (GLP-1) from canine ileal loops. *Acta Endocrinol.* **123,** 464–470.

Shimizu I., Hirota M., Obhoshi C., and Shima K. (1987) Identification and localization of glucagon-like peptide-1 and its receptor in rat brain. *Endocrinology* **121,** 1076–1082.

Suda K., Takahashi H., Fukase N., Manaka H., Tomija M., and Sasaki H. (1989) Distribution of molecular forms of glucagon-like peptides in the dog. *Life Sci.* **45,** 1793–1798.

Sugiyama K., Manaka H., Kato T., Yamatani K., Tominaga M., and Sasaki H. (1994) Stimulation of truncated glucagon-like peptide-1 release from isolated perfused canine ileum by glucose absorption. *Digestion* **55,** 24–28.

Tang-Christensen M., Larsen P. J., Göke R., Fink-Jensen A., Jessop D. S., Moller M., et al. (1996) Central administration of GLP-1 (7–36) amide inhibits food and water intake in rats. *Am. J. Physiol.* **271,** R848–856.

Thiele T. E., Van Dijk G., Campfield A., Smith F. J., Burn P., Woods S. C., et al. (1997) Central infusion of GLP-1, but not leptin, produces conditioned taste aversion in rats. *Am. J. Physiol.* **272,** R726–R730.

Thorens B. (1992) Expression cloning of the pancreatic β cell receptor for the gluco-incretin hormone glucagon-like peptide-1. *Proc. Natl. Acad. Sci. USA* **89,** 8641–8645.

Thorens B., Ponet A., Bühler L., Deng S. P., Morel P., and Widmann C. (1993) Cloning and functional expression of the human islet GLP-1 receptor. *Diabetes* **42,** 1678–1682.

Treherme J. M. and Ashford M. L. J. (1992) Extracellular cations modulate the ATP senstivity of ATP-K+ channels in rat ventromedial hypothalamic neurons. *Proc. R. Soc. Lond. Biol. Sci.* **247,** 121–124.

Turton M. D., O'Shea D., Gunn I., Beale S. A., Eduards C. M. B., Meeran K., Choi S. J., Taylor G. M., Heath M. M., Lambert P. D., Wilding J. P. H., Smith D. M., Elatei M. A., Herbert J., and Bloom S. R. (1996) A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature* **379,** 69–72.

Uttenthal O. and Blázquez E. (1990) Characterization of high-affinity receptors for truncated glucagon-like peptide-1 in rat gastric glands. *FEBS Lett.* **262,** 139–141.

Uttenthal O., Toledano A., and Blázquez E. (1992) Autoradiographic localization of receptors from glucagon-like peptide-1 (7–36) amide in rat brain. *Neuropeptides* **21,** 143–146.

Valverde I., Morales M., Clemente F., Lopez-Delgado M., Delgado E., Perea A., and Villanueva-Peñacarrillo M. (1994) Glucagon-like peptide 1: a potent glycogenic hormone. *FEBS Lett.* **283,** 7–10.

Valverde I., Mérida E., Delgado E., Trapote M. A., and Villanueva-Peñacarrillo M. L. (1993) Presence and characterization of glucagon-like peptide-1 (7–36) amide receptors in solubilized membranes of rat adipose tissue. *Endocrinology* **132,** 75–79.

Van Dijk G., Thiele T. E., Seeley R. J., Woods S. C., and Bernstein J. L. (1997) Glucagon-like peptide-1 and satiety. *Nature* **385,** 214.

Villanueva-Peñacarrillo M. L., Alcántara A. I., Trapote M. A., Clemente F., Delgado E., and Valverde I. (1994) Potent glycogenic effect of GLP-1 (7–36) amide in rat skeletal muscle. *Diabetologia* **37,** 1163–1166.

Wang T., Edwards G. L., and Baile C. A. (1996) Glucagon-like peptide-1 (7–36) amide (GLP-1) activates the hypothalamic-pituitary-adrenal (HPA) axis of rats. *Soc. Neurosci. Abstracts* **22,** 456.

Wei Y., and Mojsov S. (1995) Tissue-specific expression of the human receptor for glucagon-like peptide-1: brain, heart and pancreatic forma have the same deduced amino acid sequences. *FEBS Lett.* **358,** 219–224.

Wettergren A., Schjoldager B., Mortensen P. E., Myhre J., Christiansen J. and Holst J. J. (1993) Truncated GLP-1 (proglucagon 72–107 amide) inhibits gastric and pancreatic functions in man. *Dig. Dis. Sci.* **38,** 665–673.

Zullo J. A., Esquifino A. J., Chowder J. A., Alvarez E., Castrillion P. O., and Blázquez E. (1999) Coexpression of glucagon-like peptide-1 (GLP-1) receptor, vasopressin, and oxytocin mRNAs in neurons of the rat hypothalamic supraoptic and paraventricular nuclei: effect of GLP-1 (7–36) amide on vasopressin and oxytocin release. *J. Neurochem.*, in press.