Human glucagon-like peptides 1 and 2 activate rat brain adenylate cyclase

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Two human glucagon-like peptides, GLP-1 and GLP-2, which are coencoded with pancreatic glucagon in the preproglucagon gene, do not significantly inhibit [^{125}I]monoiodoglucagon binding to rat liver and brain membranes and do not activate adenylate cyclase in liver plasma membranes. Nevertheless, GLP-1 and GLP-2 were each found to be potent stimulators of both rat hypothalamic and pituitary adenylate cyclase. Only 30–50 pM concentrations of each peptide elicited half-maximal adenylate cyclase stimulation. Our data suggest that GLP-1 and GLP-2 may be neurotransmitters and/or neuroendocrine effectors, which would account for their high degree of sequence conservation through vertebrate evolution.

Glucagon-like peptide 1 *Glucagon-like peptide 2* *Glucagon* *cAMP* *Pituitary* *Hypothalamus* *Liver plasma membrane*

1. INTRODUCTION

Glucagon, a 29 amino acid peptide hormone is well-known for its important role in peripheral metabolic processes [1]. As is true for other peptide hormones, the synthesis of glucagon is thought to involve a larger precursor [2]. Although the pancreas is the most abundant source of glucagon, larger forms of glucagon have also been found in intestine, stomach, brain and salivary glands [3]. Inasmuch as pancreatic preproglucagon contains the sequences of several intestinal glucagon-containing polypeptides, it has been suggested that tissue-specific processing of a common precursor could generate an array of peptides [4].

Recently, the structures of hamster [4] and bovine [5] pancreatic preproglucagon were deduced from the corresponding cloned cDNA. Furthermore, the structure of human preproglucagon was determined from a genomic sequence [6]. The amino acid sequences of the 3 glucagon precursors were found to be similar [6]. The 180 amino acid mammalian preproglucagon codes for a signal peptide, an amino-terminal glicentin-related pancreatic polypeptide (GRPP), pancreatic glucagon, and two glucagon-like peptides called GLP-1 and GLP-2 arranged in tandem. Except for the signal peptide, the other coencoded peptides are flanked by dibasic residues which represent putative processing sites. The sequences of two non-allelic anglerfish pancreatic glucagon precursors have also been determined and their organization found to be similar except for the absence of GLP-2 [7].

GLP-1 (37 amino acids) and GLP-2 (34 amino acids) are related but not identical to other members of the glucagon-secretin family of gastrointestinal hormones [4]. The two glucagon-like peptides have been described as 'cryptic' because their isolation and physiological properties had not been reported. The GLP-1 sequence possesses extensive homology between hamster and anglerfish and is identical in the 3 mammalian preproglucagons reported. Amino acid sequence homology of GLP-2 between human and hamster is 91.2% and between human and bovine it is 85%.

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Abbreviations: GLP-1, glucagon-like peptide 1; GLP-2, glucagon-like peptide 2

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The high degree of sequence conservation of GLP-1 and GLP-2 has suggested that these peptides have important physiological functions [4,6,8]. This and the structural similarity to glucagon prompted us to investigate the ability of GLP-1 and GLP-2 to inhibit [^{125}I]-monoiodoglucagon binding and to activate adenylate cyclase in liver plasma membranes. We have identified, localized and characterized glucagon receptors in rat brain and pituitary [9]. Therefore, we also examined the effect of the two peptides on rat hypothalamic and pituitary membranes.

2. MATERIALS AND METHODS

Chromatographically pure, synthetic human GLP-1 (36 amino acids with amidated C-terminal) and GLP-2 (34 residues) were purchased from Peninsula (Belmont, CA). Porcine glucagon (a gift from Eli Lilly and Co., Indianapolis, IN) was purified, iodinated using IODOGEN, and [^{125}I]-monoiodoglucagon was purified as described in [10]. Partially purified liver plasma membranes and hypothalamic as well as pituitary homogenates were prepared as described [9]. Competitive binding and adenylate cyclase assays were performed as in [9].

3. RESULTS

As shown in fig.1 (top), with membrane preparations from liver and brain, glucagon is able to displace [^{125}I]-monoiodoglucan at half-maximum concentrations of 1 and 5 nM, respectively. In neither preparation (4 separate assays) was GLP-1 or GLP-2 able to displace the labelled glucagon except to a minimal extent at much higher concentrations (>1 μM) in liver. As shown in fig.1 (bottom), when GLP-1 and GLP-2 were assessed for their abilities to activate adenylate cyclase in liver membranes, neither peptide was able to activate cyclase to the extent or in the concentration range at which glucagon itself is effective. However, as shown in fig.2, with hypothalamus and pituitary membrane preparations both GLP-1 and GLP-2 activate adenylate cyclase 2-fold over basal activity in the presence of 1.0 mM theophylline. The half-maximum concentration for activation is low, near 30–50 pM for GLP-1 and GLP-2, in contrast to 5 nM for glucagon itself [8]. As further seen in fig.2, at peptide concentrations greater than those which produce maximum activation, cyclase activity falls rapidly to near basal levels for both GLP-1 and GLP-2 with both hypothalamic and pituitary membranes. K_i values are ~0.8 nM for GLP-1 and ~4 nM for GLP-2, concentrations very near those for half-maximum activation by glucagon.

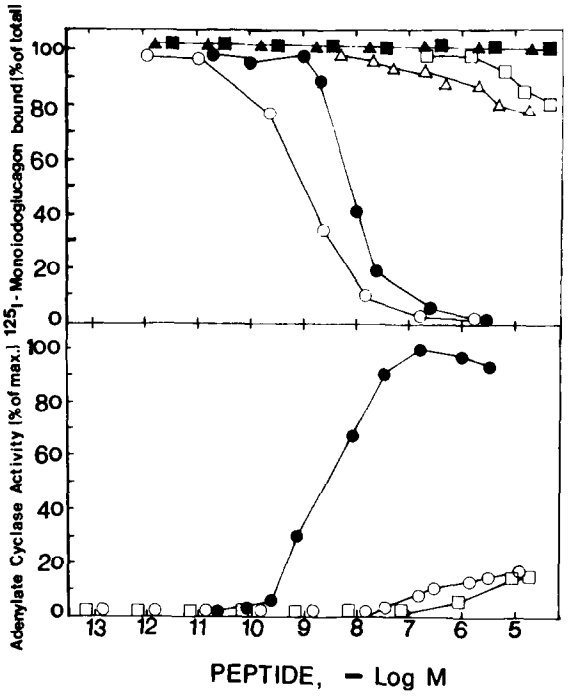

Fig.1. Typical competition and dose response curves with glucagon and glucagon-like peptides. Top: displacement of [^{125}I]-monoiodoglucagon bound to liver plasma membranes by (○) glucagon, (△) GLP-1 and (□) GLP-2. Similar plots for hypothalamic and pituitary pooled homogenates with (●) glucagon, (▲) GLP-1 and (■) GLP-2. Bottom: activation of liver plasma membrane adenylate cyclase by (●) glucagon, (○) GLP-1 and (□) GLP-2. Results are expressed as percentage of maximal activity attained by glucagon. Basal activity was 0.95 nmol cAMP formed per mg protein in 10 min. ~3-fold activation over basal levels was seen with glucagon. SD of each point performed in triplicate was <5%. All assays were performed at least 3 times. For glucagon k_{act} = 3.7 nM.

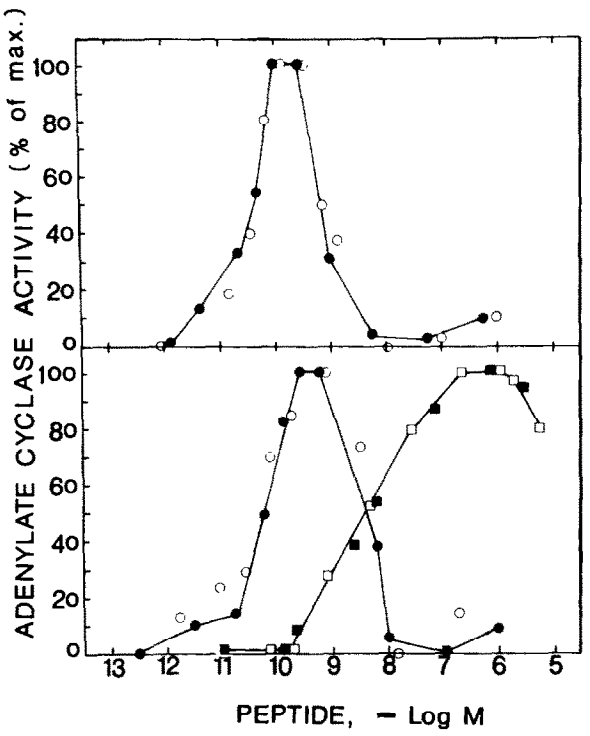

Fig.2. Typical dose response curves for the activation of brain adenylate cyclase. Top: activation by GLP-1 in rat (●) hypothalamic and (○) pituitary homogenates. Bottom: activation by (●, ○) GLP-2 and (■, □) glucagon in (●, ■) hypothalamic and (○, □) pituitary homogenates. Basal activity was ~0.5 and 0.15 nmol cAMP formed per mg wet wt of tissue for hypothalamic and pituitary homogenates, respectively. Activation over basal levels was ~2-fold. Other details as in legend to fig.1.

4. DISCUSSION

Our data clearly indicate that GLP-1 and GLP-2 do not interact with glucagon receptors in rat liver, pituitary or hypothalamic membranes at physiologically relevant concentrations (fig.1). However, the two glucagon-like peptides are very potent in activating adenylate cyclase in both rat hypothalamic as well as pituitary membranes at concentrations significantly lower (30–50 pM) than that seen for glucagon (5 nM). High dose inhibition of adenylate cyclase seen with GLP-1 and GLP-2 has been reported earlier for a number of activators of adenylate cyclase including glucagon, secretin, β-adrenergic agonists and prostaglandins [11,12].

There have been reports of the isolation of large molecular forms of glucagon from the CNS of rat [13] and dog [14,15]. In rat brain [13], two major polypeptides of molecular mass 8 and 12 kDa, and representing incompletely processed forms of preproglucagon were found. The sizes of these peptides and their selective glucagon-like immunoreactivities were similar to intestinal glucagon-related peptides [13,16]. The 8-kDa protein contains the amino acid sequences of GRPP and pancreatic glucagon. The size, biochemical and immunological properties of the 12-kDa protein suggests that it encompasses GLP-1 in addition to GRPP and pancreatic glucagon [4]. These studies provide indirect evidence for processing of preproglucagon to yield glucagon and glucagon-like peptides and suggest that local synthesis of the peptides may occur in brain.

Because GLP-1 and GLP-2 activate rat hypothalamic and pituitary adenylate cyclase at low concentrations and do not cross-react with glucagon receptors in both CNS and liver, it appears that the two glucagon-like peptides have their own receptors and may well be novel neurotransmitters and/or neuromodulators. This may account for the highly conserved amino acid sequences of the two peptides. The physiologic role of GLP-1 and GLP-2 in the CNS remains enigmatic and requires further investigation.

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