

Effect of Corticotropin-like Intermediate Lobe Peptide on Pancreatic Exocrine Function in Isolated Rat Pancreatic Lobules

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Abstract. Naturally occurring derivatives of pro-opiomelanocortin (POMC) have been identified in various extra-pituitary sites, including the endocrine and exocrine pancreas. Corticotropin-like intermediate lobe peptide (CLIP=ACTH₁₈₋₃₉), a naturally occurring derivative of POMC, has been suggested to be an insulin secretagogue. To determine whether CLIP might also affect the exocrine pancreas, we measured its effect on amylase secretion and protein synthesis and secretion in isolated rat pancreatic lobules. Lobules were dual-pulsed with trace amounts of ¹⁴C- and ³H-leucine, both in the presence and absence of CLIP (10⁻⁹–10⁻⁶ M), using a technique that permitted the labeling of both the synthetic and secretory compartments. The effect of CLIP on protein synthesis was determined by comparing ³H-leucine incorporation into lobules with and without CLIP. The secretory effect of CLIP was determined by measuring (a) secreted ¹⁴C-labeled protein as a percent of total incorporated radiolabeled protein, and (b) amylase release into incubation medium. The effect of CLIP on amylase release was compared with that of secretin, cholecystokinin-octapeptide, and carbamylcholine. To localize the biologically active region of CLIP, we similarly studied synthetic ACTH₂₅₋₃₉. We demonstrated that CLIP stimulates amylase and protein secre-

tion in a dose-dependent manner and is of similar potency to secretin and carbamylcholine. This effect appears to require the ACTH₁₈₋₂₄ region of CLIP and results from stimulus-secretion coupling rather than augmented protein synthesis. We also confirmed the presence of immunoreactive-adrenocorticotrophic hormone (IR-ACTH) in rat pancreatic extract using a COOH-terminally directed antibody to ACTH₁₋₃₉ and demonstrated that this IR-ACTH co-eluted with synthetic CLIP. These findings suggest that CLIP might be an endogenous modulator of pancreatic exocrine function.

Introduction

It has previously been proposed that certain peptides derived from the common precursor pro-opiomelanocortin (POMC)¹ and typically associated with the pituitary may also be normal constituents of other tissues, including the brain (1), gastric antrum (2), colon, liver, heart (3), small intestine, and pancreas (4). Although brain has been shown to produce POMC peptides (5), the source of similar peptides found in other extra-pituitary sites has not been clearly established.

Reports have begun to characterize the potential physiological roles of POMC peptides in extra-pituitary sites. In pancreas, it has been suggested that β -endorphin affects glucose metabolism by influencing glucagon (6), somatostatin (7), and/or insulin (8) secretion from pancreatic islets. Other investigators have proposed that corticotropin-like intermediate lobe peptide (CLIP=ACTH₁₈₋₃₉), a naturally occurring derivative of ACTH₁₋₃₉, or a similar peptide is an insulin secretagogue (9).

Because certain gastrointestinal peptides are known to affect both pancreatic endocrine and exocrine secretion, and

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1. Abbreviations used in this paper: CLIP, corticotropin-like intermediate lobe peptide; IR-ACTH, immunoreactive adrenocorticotrophic hormone; POMC, pro-opiomelanocortin.

immunocytochemical staining for ACTH has been localized to both endocrine and exocrine pancreas (10), we sought to determine whether CLIP affected the exocrine pancreas by assessing its effect on pancreatic protein synthesis and protein and amylase secretion in isolated rat pancreatic lobules.

Methods

Materials. Sprague Dawley rats (250–300 g) were obtained from Zivic-Miller Laboratories, Inc., Allison Park, PA. Synthetic CLIP, synthetic porcine secretin (pentacetate salt), cholecystokinin-octapeptide, Hepes, type I-S trypsin inhibitor, and carbamylcholine were obtained from Sigma Chemical Co., St. Louis, MO. Synthetic ACTH_{25–39} was generously provided by Ciba-Geigy Corp., Ardsley, NY. Bovine serum albumin (BSA), Cohn fraction V was obtained from Miles Laboratories, Inc., Elkhart, IN. Essential and nonessential amino acids were obtained from ICN Nutritional Biochemicals, Cleveland, OH. Brij-35, Spectraphor cellulose dialysis tubing, ScintiGest tissue solubilizer, and ScintiVerse scintillation fluid were obtained from Fisher Scientific Co., Pittsburgh, PA. L-¹⁴C(U)-Leucine (330 mCi/mmol) and L-3,4,5-³H(N)-leucine (134 Ci/mmol) were obtained from New England Nuclear, Boston, MA. Diphenylamine was obtained from Eastman Laboratory and Specialty Chemicals, Rochester, NY. Amylase assay reagents were obtained from Beckman Instruments, Inc., Fullerton, CA.

Immunoassay and chromatographic characterization. Adult rat pancreatic extract was obtained and immunoassayed using similar methods for extraction and radioimmunoassay as previously described (11). The ACTH antisera (kindly provided by Dr. John Kendall, Portland, OR) is directed toward the carboxy-terminal portion of ACTH_{1–39}, and reacts approximately on an equimolar basis with ACTH_{1–39}, ACTH_{18–39}, and ACTH_{25–39}.

Gel filtration chromatographic characterization was performed by applying the extract to a G-25 Sephadex column (fine; 0.9 × 45 cm) and eluting the material with a dissociating eluant containing 8 M urea, 0.05 M sodium phosphate, 0.25% BSA, 0.5% mercaptoethanol, and 0.02% merthiolate, pH 7.5.

Pancreatic lobule dual-pulse labeling technique. Rat pancreatic lobules, prepared after the method of Scheele and Palade (12), were placed on fine mesh grids at the base of cylindrical incubation chambers. They were suspended in a solution containing 125 mM NaCl, 2.5 mM CaCl₂, 4.6 mM KCl, and 1.16 mM MgSO₄, buffered with 25 mM Hepes, and supplemented with 1 mg/ml glucose and 100 µg/ml BSA at 37°C, 287 mOsm, pH 7.4, 90 oscillations/min under a 95%O₂/5%CO₂ atmosphere. The lobules were initially pulsed for 15 min with a trace amount of L-¹⁴C(U)-leucine, washed, and subsequently incubated for 45 min in a chase solution containing 24.5 mM Hepes, 98 mM NaCl, 6 mM KCl, 2.5 mM NaH₂PO₄, 5 mM Na pyruvate, 5 mM Na fumarate, 5 mM glutamate, 2.5 mM CaCl₂·2H₂O, 1 mM MgCl₂·6H₂O, 2 mM glutamine, 11.5 mM glucose, 0.2% (wt/vol) BSA, and 0.01% (vol/vol) essential vitamin and amino acid mixture (including unlabeled leucine), under conditions identical to the initial pulse. This chase moved the newly synthesized ¹⁴C-labeled secretory proteins from rough endoplasmic reticulum to the storage granules before exposure to secretagogues. The lobules were pulsed a second time, with a different radiotracer, L-3,4,5-³H(N)-leucine, under incubation conditions identical to the first pulse and in the presence of either CLIP (10^{–9}–10^{–5} M) or ACTH_{25–39} (10^{–9}–5 × 10^{–6} M). The lobules were subsequently incubated with the same peptide for an additional 180 min. For each experiment, lobules treated under identical conditions, but not exposed to either peptide, served as controls.

Preparation of medium and tissue homogenate for radiolabeled protein assay. Aliquots of each hourly sample of incubation medium and the tissue homogenate were dialyzed against tap water, 0.9% NaCl with 2.05 mM cold leucine and deionized water, precipitated with 10% TCA, resolubilized, and counted for both ¹⁴C- and ³H-radioactivity. Efficiency determinations were made using internal and external standardization methods. Radiolabeled protein activity was expressed per microgram tissue DNA.

Amylase determinations. Aliquots of both incubation medium and lobule homogenates were measured spectrophotometrically for amylase activity using the enzymatic amylase-DS reagent system (Beckman Instruments, Inc.) after the method of Pierre et al. (13). Amylase activity was expressed per microgram tissue DNA.

Definitions. Protein synthesis was defined as the total incorporation of ³H-leucine into TCA-precipitable proteins (tissue plus medium) per microgram tissue DNA. Protein synthesis in lobules exposed to either CLIP or ACTH_{25–39} was expressed as a percent of control lobules. Protein secretion was defined as the amount of ¹⁴C-labeled protein released into the medium as a percent of total incorporated ¹⁴C-radioactivity. Amylase secretion was defined as that percentage of total amylase (tissue plus medium) released into the medium during a specific period of incubation, and was expressed as percent stimulation above control lobules.

Statistics. The effects of various peptides on experimental lobules were compared with control lobules using the *t* test for unpaired data. The experimental results were expressed as mean ± SEM for each group of lobules.

Results

Immunoassay and chromatographic characterization of rat pancreatic extract. Adult rat pancreas contained 195 pg IR-ACTH/mg protein. Gel filtration chromatographic characterization of rat pancreatic extract demonstrated a peak of ACTH immunoreactivity co-eluting with synthetic CLIP (Fig. 1).

Effect of clip and ACTH_{25–39} on amylase secretion. Increasing concentrations of CLIP produced a significant dose-dependent stimulation of amylase release (Fig. 2). When the potency of CLIP, defined as that molar concentration producing a 100%

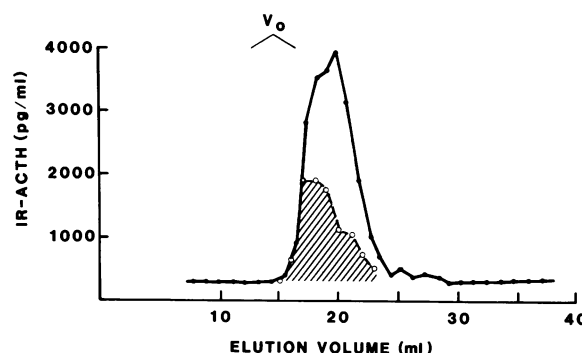


Figure 1. IR-ACTH in chromatographic separation (G-25 Sephadex; fine) of rat pancreatic extract and synthetic CLIP (shaded area). IR-ACTH was determined with an antiserum to the carboxy-terminal portion of ACTH_{1–39}. ●, pancreatic extract; ○, synthetic CLIP.

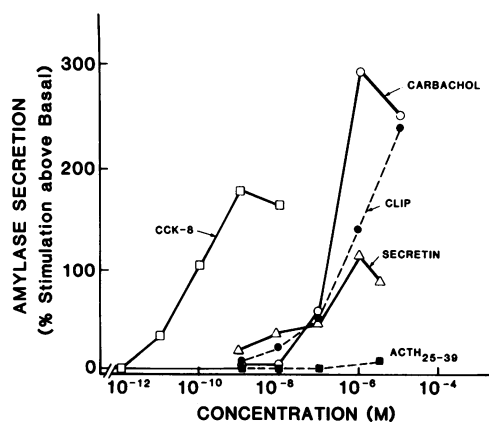


Figure 2. Effect of CLIP (●), ACTH₂₅₋₃₉ (■), cholecystokinin-octapeptide (□), secretin (Δ) and carbamylcholine (○) on amylase secretion from isolated rat pancreatic lobules. Lobules were incubated for 60 min at 37°C with the concentrations of substances indicated. For each series of experiments, lobules were incubated in the absence of secretagogue and the amylase release from these lobules represented basal secretion. The secretagogue-induced stimulation of amylase secretion was expressed as the mean percentage stimulation for 3–6 experiments for each peptide concentration.

stimulation of amylase release over control, was compared with known pancreatic secretagogues, CLIP was less potent than cholecystokinin-octapeptide, but showed a similar potency to secretin and carbamylcholine. Although we did not use a concentration of CLIP that allowed us to determine maximal amylase stimulation, it would appear to be greater than that of all substances studied, with the possible exception of carbamylcholine. In contrast, ACTH₂₅₋₃₉, the carboxy-terminal fragment of CLIP, failed to stimulate amylase release.

Effect of CLIP and ACTH₂₅₋₃₉ on radiolabeled protein synthesis and secretion. Exposure of isolated rat pancreatic lobules to increasing concentrations of CLIP (10^{-9} – 10^{-5} M) resulted in a dose-dependent increase in the secretion of ¹⁴C-labeled protein into the incubation medium (Fig. 3). Increments over control secretion at 60 min were significant at both 10^{-6} M ($P < 0.01$) and 10^{-5} M ($P < 0.001$) CLIP. The increased secretion produced by 10^{-7} M CLIP became significant ($P < 0.001$) at 120 min, and persisted at 180 min, as did secretion stimulated by 10^{-6} M CLIP. Lower concentrations of CLIP (10^{-9} and 10^{-8} M; data not shown) failed to augment protein secretion during the 180-min incubation period.

To characterize further the region of CLIP mediating this protein secretion, we studied ACTH₂₅₋₃₉, a COOH-terminal fragment of CLIP. This fragment failed to stimulate protein secretion at concentrations ranging from 10^{-9} to 5×10^{-6} M (Fig. 3).

Effect of CLIP and ACTH₂₅₋₃₉ on radiolabeled protein synthesis. Neither CLIP nor ACTH₂₅₋₃₉ significantly altered the rate of pancreatic export protein synthesis at any concentration studied. ³H-Leucine incorporation into lobules exposed

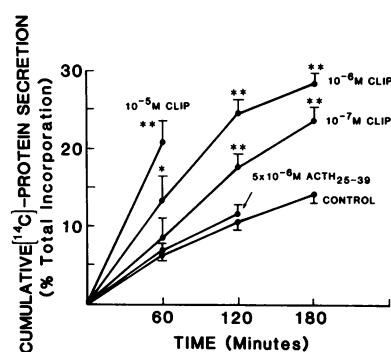


Figure 3. Effect of CLIP and ACTH₂₅₋₃₉ on radiolabeled protein secretion from isolated rat pancreatic lobules. Lobules were incubated for 180 min at 37°C with the concentrations of peptides indicated. The cumulative percentage of ¹⁴C-labeled protein secreted into the incubation medium is expressed as mean±SEM for three to six experiments at each peptide concentration. Protein secretion from lobules stimulated with 10^{-9} and 10^{-8} M CLIP (data not shown) did not differ from control lobules. Asterisks denote stimulation significantly greater than controls; * $P < 0.01$, ** $P < 0.001$.

to 10^{-9} and 10^{-5} M CLIP was 91 ± 8 and $107 \pm 6\%$ of control lobules, respectively, while incorporation into lobules exposed to 10^{-9} and 5×10^{-6} M ACTH₂₅₋₃₉ was 86 ± 14 and $94 \pm 3\%$, respectively.

Discussion

We have demonstrated that CLIP stimulates both protein and enzyme secretion from isolated pancreatic lobules. These data provide the first evidence that CLIP=ACTH₁₈₋₃₉, a naturally occurring derivative of ACTH₁₋₃₉, is a biologically active peptide in pancreas. Before these observations and those of a recent preliminary report showing that CLIP modulates pituitary hormone secretion (14), this POMC derivative had not been recognized as having any biologic activity. Additional evidence supporting a physiological role for CLIP in the regulation of pancreatic exocrine protein secretion is suggested by: (a) our identification of immunoassayable ACTH in pancreatic extract and our demonstration of a peak of ACTH immunoreactivity co-eluting with synthetic CLIP; (b) the recent radioautographic demonstration of specific binding of labeled-ACTH to exocrine pancreas in vivo (15), and (c) our observation that CLIP is equipotent to other recognized pancreatic secretagogues. The effect of CLIP on enzyme release appears to occur through stimulus-secretion coupling rather than as a result of increased protein synthesis. In addition, this activity seems to require the amino-terminal portion of the peptide since exposure of lobules to ACTH₂₅₋₃₉ altered neither radiolabeled protein nor amylase secretion.

These observations, coupled with our more recent report that ACTH₁₋₁₈ also stimulates pancreatic amylase secretion in vitro (16), suggest potentially important physiological roles for

POMC peptides in the modulation of pancreatic function. Although a recent report (17) demonstrated that ACTH₂₂₋₃₉ possessed insulinotropic activity while CLIP was inactive at $\sim 4 \times 10^{-10}$ M, the physiological significance of these and other POMC-related peptides in the regulation of both pancreatic exocrine and endocrine function remains to be established.

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