Evidence for the Paracrine Action of Islet-Derived Corticotropin-Like Peptides on the Regulation of Insulin Release

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In view of recent evidence for the endogenous synthesis of proopiomelanocortin (POMC) by pancreatic islets, we have assessed (1) the release of POMC-derived corticotropin (ACTH)-like peptides (ACTH-LP) from isolated perifused rat islets, and (2) the potential paracrine modulatory effect on insulin output of these putative secretagogues. Islets perifused at a glucose concentration of 3.3 mmol/L secreted ACTH-LP at 0.15 ± 0.005 ng/islet/10 min, which was increased by 17-fold at 16.7 mmol/L glucose. Islets statically incubated with different concentrations of medium glucose plus synthetic $^{1-39}$ACTH at 55 pmol/L showed a significant increase of insulin release at 8 (by 79%) and 16 (by 119%) mmol/L glucose, but not at 4 mmol/L. To determine the possible *cis*-directed effects of these endogenously released islet ACTH-LP on insulin secretion, we either blocked their biological action by immunoneutralization with an ACTH-specific antiserum or prevented their receptor interaction by addition of the ACTH-inhibiting polypeptide (CIP) to the incubation medium. In the presence of 16.7 mmol/L glucose, the rate of insulin output decreased by approximately 25% upon exposure to the antiserum and by approximately 50% in the presence of CIP. The foregoing observations would therefore suggest that both (1) the elaboration of ACTH-LP by isolated perifused islets and (2) the stimulation of islet insulin release by exogenous $^{1-39}$ACTH in static incubation occur as a function of glucose concentration in the incubation medium, and that (3) the newly-secreted endogenous ACTH-LP operate in a *cis* mode to enhance islet insulin output in a manner analogous to that of exogenously added ACTH species. These results strongly support the view that islet-elaborated ACTH-LP are important physiological paracrine modulators of insulin secretion.

PROOPIOMELANOCORTIN (POMC), the 265–amino acid molecular precursor of several pituitary peptide hormones, has been detected in diverse mammalian organs,[1] including normal islets of Langerhans in the rat.[2] Although the identity of the particular cellular elements responsible for elaboration of POMC in the latter source has not yet been determined,[3] the presence of POMC-specific mRNA within these glands[2] constitutes cogent evidence for the endogenous synthesis of that protein by islet tissue. This putative production of POMC by the endocrine pancreas, in turn, appears to be under hormonal and metabolic regulation, since dexamethasone treatment of intact rats diminished the quantity of POMC mRNA within this endocrine complex,[3] and the release of POMC itself from isolated islets in vitro was stimulated by addition of glucose to the incubation medium.[4] Such observations provided initial circumstantial evidence for the participation of POMC as an intraislet, and thus paracrine, hormonal regulator. Although the modulatory role of the POMC molecule itself within pancreatic islets is still unclear, several smaller peptides derived from it have recently been shown to have a significant effect on insulin secretion,[5-7] and among these species, exogenous corticotropin (ACTH) in particular has long since been known to stimulate pancreatic insulin output directly.[8-12]

We have recently shown that (1) isolated rat islets statically incubated with glucose release nascent ACTH-like peptides (ACTH-LP) as a function of glucose concentration in the medium (3.3 *v* 16.7 mmol/L),[13] (2) a synthetic ACTH consisting of the entire 39–amino acid residues ($^{1-39}$ACTH) stimulates insulin secretion in the presence of low glucose (3.3 mmol/L) in a dose-dependent manner (from 50 to 500 pg/mL), and (3) the increase is accompanied by an increment in the cytosolic concentration of Ca^{2+} in B cells.[14] However, this elevation in cytosolic Ca^{2+} does not result from a change in voltage-dependent Ca^{2+} channels, but rather appears to be secondary to an inhibition of islet Ca^{2+}-adenosine triphosphatase on the part of ACTH.[15] It remains to be determined whether endogenously generated ACTH exhibits these actions and, if so, under what circumstances.

In the present study, we have addressed this last question in the following manner. We first reexamined in greater detail the profile of ACTH-LP production by isolated perifused islets in response to different concentrations of glucose in the medium. We then used two separate experimental approaches to gain initial evidence that these same nascent POMC derivatives modulate islet insulin secretion themselves in a *cis* fashion—ie, they act directly within their own tissue of origin as paracrine hormonal regulators. To this end, we attempted to block their putative endogenous action in situ by exposing islets either to the same antibody against ACTH as was used for its assay or to the ACTH-inhibiting polypeptide (CIP) in the presence of high glucose. We finally confirmed, as well, that the $^{1-39}$ACTH used in our previous studies elicits an enhancement of islet insulin output at concentrations within the physiologic range, provided that sufficient levels of glucose are present in the incubation medium.

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MATERIALS AND METHODS

Islet Harvest

Male Wistar rats weighing 300 to 350 g were used as a source of islets. They were fed ad libitum and kept in a temperature-controlled environment with a 12-hour light/dark cycle. Islets were prepared by means of collagenase treatment as outlined in detail previously.[16] After enzymatic digestion, the pancreases were repeatedly washed and the islets rapidly hand-picked with siliconized glass pipettes under a dissecting microscope.

Perifusion

Batches of 120 islets were perifused as described previously[17] in chambers containing 1 mL Bio-Gel P2 (100 to 200 mesh; Bio-Rad Laboratories, Richmond, CA) in Krebs-Ringer bicarbonate (KRB) buffer supplemented with 20 mmol/L HEPES, 2 mg/mL bovine serum albumin (BSA), 100 IU/mL penicillin, 100 μg/mL streptomycin, 400 kallikrein-inhibitor units (KIU)/mL aprotinin (Trasylol; Bayer, Leverkusen, Germany), and either 3.3 or 16.7 mmol/L glucose under an atmosphere of 95% oxygen–5% CO_2 at pH 7.4. The perifusion chambers were installed in an isothermal box at 37°C; each chamber was perifused with buffer at a flow rate of 0.1 mL/min, and 1-mL fractions of the perfusate were collected for assay every 10 minutes. This low flow rate was deliberately selected to obtain a sufficient concentration of ACTH-LP for facile and accurate radioimmunoassay. Each perifusion study was undertaken with a paired control and repeated five times. Previous experience has demonstrated that islets retain the ability to modify insulin secretion, as a result of the stimulus of glucose or other secretagogues, farther from the time period currently used for perifusion or static incubation.[17,18]

Experimental Protocols

Measurement of ACTH-LP and insulin co-release. To assess the pattern of ACTH-LP release in relation to insulin secretion, 120 islets were placed in each perifusion chamber. Such a large number was necessary for accuracy in the assay, since only small quantities of ACTH-LP were released from each chamber (see the Results). Islets were first preequilibrated for 40 minutes with buffer containing 3.3 mmol/L glucose, and this same perifusion medium was then maintained for an additional 29 minutes, at the end of which (time 0) an initial sample was taken. The perifusion medium was next changed to one containing glucose at a concentration of 16.7 mmol/L for a further 20 minutes of incubation before a final portion of the perifusate was removed for analysis. After storage at −20°C, ACTH-LP and insulin levels were measured in aliquots of the used media by radioimmunoassay.

To ensure that the ACTH-LP secreted were not being inactivated during the standard experimental procedure, we performed a control study to test their stability under the conditions of perifusion. Results of this pilot experiment reassured us that more than 95% of these peptides could be recovered in the presence of 400 KIU/mL of the protease inhibitor aprotinin.

For ACTH-LP radioimmunoassay, we used a rabbit antibody against the "midportion" of the ACTH molecule that cross-reacted only negligibly (<0.1%) with α-melanocyte–stimulating hormone (α-MSH), ACTH-like intermediary peptide, or any of the other known POMC cleavage products.[19] This assay system uses either $^{1\text{-}39}$ACTH or $^{1\text{-}24}$ACTH (Peninsula, Palo Alto, CA) labeled with ^{125}I as a tracer[20] along with polyethyleneglycol for separation of the free from the bound fractions. No interference from either insulin or proinsulin was detected under these conditions.

For insulin radioimmunoassay, we used a commercial insulin antibody, a rat insulin standard, and a highly purified ^{125}I-labeled porcine insulin. All these reagents were kindly provided by Dr Lise Heding (Novo Nordisk, Bagsværd, Denmark). Our radioiodination procedure involves the use of polyacrylamide gel electrophoresis for isolation of monoiodinated hormone from more highly labeled species,[21] and the assay itself uses a charcoal-dextran mixture for separation of the free from the bound insulin.[22] The possible interference of ACTH in the insulin assay was also studied by adding synthetic $^{1\text{-}39}$ACTH (80 pg/mL) either to the insulin standard curve or to samples of the perifusion medium. ACTH produced a slight noncompetitive displacement of the ^{125}I-insulin tracer that only became apparent at the lowest insulin concentration included in our standard curve. Consequently, all samples were diluted appropriately to obtain insulin levels far above the range of sensitivity to interference, and parallel controls were performed both with and without addition of known amounts of ACTH. In separate control trials, we also verified that the amounts of ACTH antibody or CIP used in the experiments described below did not affect results of the insulin assay.

Insulin release in the presence of added synthetic $^{1\text{-}39}$ACTH. Five islets per tube were statically incubated in 0.6 mL KRB containing 10 mg/mL BSA and 400 KIU/mL aprotinin for 60 minutes at 37°C as described in detail previously,[23] to assess the effects of added ACTH on insulin secretion. The incubation buffer contained either 4, 8, or 16 mmol/L glucose in the presence or absence of 55 pmol/L synthetic $^{1\text{-}39}$ACTH. ACTH, when present, was dissolved in incubation buffer immediately before use.

Effect of endogenously generated ACTH-LP on insulin release. For this study, each experimental group, consisting of five islets apiece, was statically incubated for 60 minutes at 37°C in 0.6 mL KRB containing 10 mg/mL BSA, 400 KIU/mL aprotinin, either 3.3 or 16.7 mmol/L glucose, and the factor to be tested, before harvesting for insulin assay as detailed earlier. In one pair of static incubations, the medium was supplemented with either rabbit antiserum to ACTH (ACTH-As) or nonimmune rabbit serum (NRS), while in another set the medium included either 55 μmol/L CIP (Bachem, Torrance, CA) or buffer alone. ACTH-As was the same antiserum used for radioimmunoassay of ACTH-LP, and both this preparation and the NRS were present at a final dilution of 1:700. In a control experiment, we tested the maximal antigen-binding capacity of this concentration of ACTH-As using the aforementioned ^{125}I-labeled porcine ACTH, and found that only 59% of the labeled peptide was bound. Although this figure would indicate that ACTH-As diluted to this extent was probably incapable of complexing with all the nascent ACTH-LP generated by the experimental static incubations, because of the scarcity of the antiserum available to us, we were nevertheless forced to use this titer to test insulin output rather than a more concentrated antibody preparation. The inhibition data we observed should thus be considered an underestimate by some 40% of the maximum value potentially obtainable in the presence of an ACTH-As of greater strength.

Statistical Analysis

The data obtained approximated a normal distribution, and results are presented as the mean ± SE. Effects of the various experimental peptides were compared with the data obtained with paired control tubes run in parallel by means of repeated-measures ANOVA and Student's *t* test. Throughout these experiments, a *P* value of less than .05 was regarded as statistically significant.

RESULTS

Hormone Release by Islets From Control Rats

ACTH-LP immunoreactive material was detectable in the perifusate of rat islets in the presence of low glucose

(3.3 mmol/L), with the mean release rate being 0.15 ± 0.005 pg/islet/10 min during the initial 30-minute period (Fig 1B). However, once the glucose concentration was increased to 16.7 mmol/L (time 0), this modest ACTH-LP secretion became augmented by 17-fold, to a level of 2.6 ± 0.7 pg/islet/10 min for the remaining 20 minutes of incubation. This response to the elevated glucose concentration was highly significant ($P < .005$) by repeated-measures ANOVA.

Basal insulin release from control rat islets was 0.07 ± 0.025 ng/islet/10 min, about 400 times greater on a molar basis than the ACTH-LP output (12 *v* 0.030 pmol/L, respectively). Insulin secretion was increased by 3.2-fold to 0.22 ± 0.04 ng/islet/10 min after the shift to the higher glucose level ($P < .001$; Fig 1A).

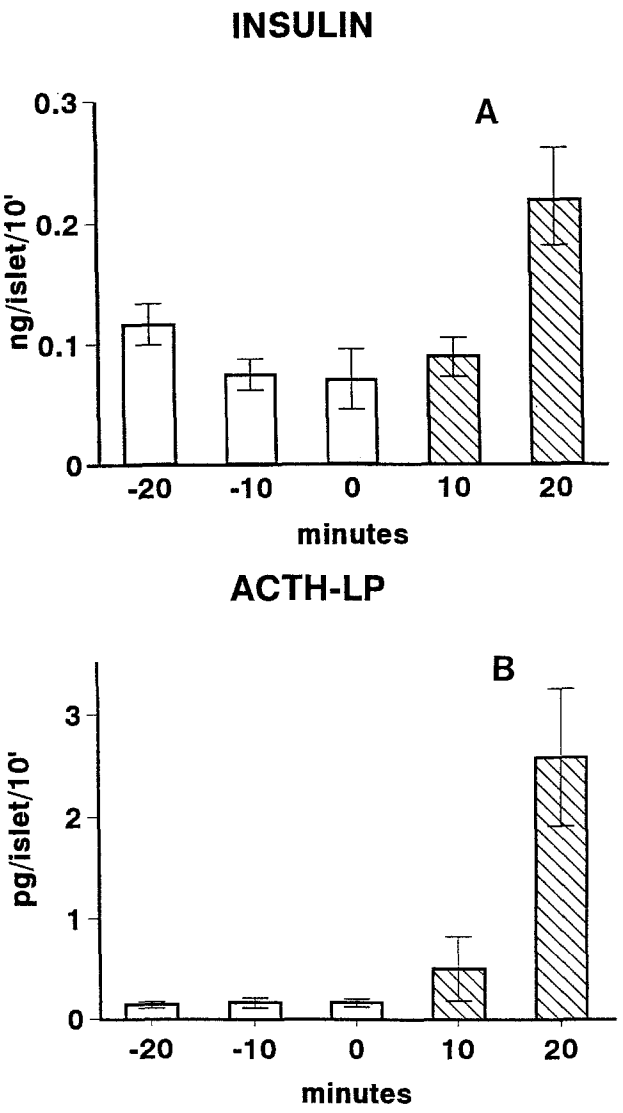

Fig 1. Secretion of insulin (A) and ACTH-LP (B) from isolated rat islets perifused first with 3.3 and then with 16.7 mmol/L glucose. Groups of 120 islets were perifused. Each time point on the abscissa in minutes demarcates the conclusion of a 10-minute interval during which aliquots of the used perifusion media were harvested for assay of hormone levels. For the first 3 of these periods, the medium contained 3.3 mmol/L glucose (□); for the last two, this concentration was shifted to 16.7 mmol/L (▨). Each ordinate value represents the mean ± SEM hormone output in pg/islet/10 min from 5 replicate samples (brackets). Insulin and ACTH-LP released at 20 minutes were significantly above their corresponding basal values ($P < .005$ and $P < .001$, respectively).

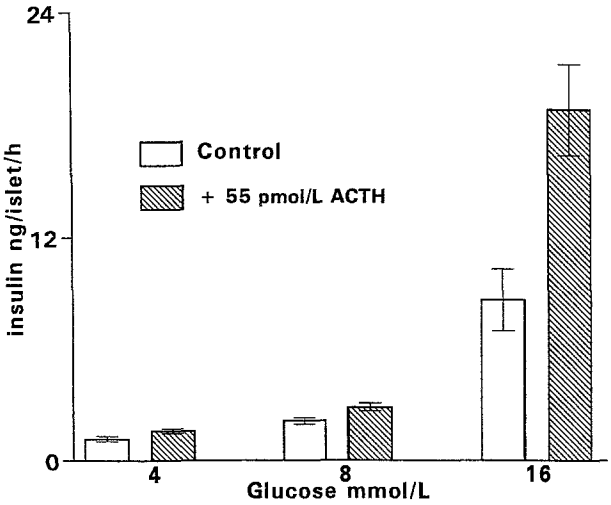

Fig 2. Insulin release in response to different concentrations of glucose in either the presence or the absence of 55 pmol/L $^{1\text{-}39}$ACTH. Each bar represents the mean ± SEM (brackets) of 20 determinations. The increase in insulin secretion upon static incubation with ACTH was significant only at glucose levels of 8 and 16 mmol/L ($P < .01$ and $P < .005$, respectively).

Effect of Added $^{1\text{-}39}$ACTH on Insulin Release

Insulin release was 0.90 ± 0.10 ng/islet/60 min in the presence of 4.0 mmol/L glucose (Fig 2), increasing to 2.1 ± 0.17 ng/islet/60 min and 8.6 ± 1.7 ng/islet/60 min at glucose concentrations of 8 and 16 mmol/L, respectively. Addition of 55 pmol/L ACTH augmented the elevated insulin output seen at 8 and 16 mmol/L glucose to values of 2.86 ± 0.21 and 18.85 ± 2.43 ng/islet/60 min (by 36% [$P < .01$] and 119% [$P < .005$], respectively). By contrast, in the presence of 4 mmol/L glucose, ACTH produced a slight but nonsignificant increase of insulin secretion.

Rat Islet ACTH Immunoneutralization In Situ

In the experiment shown in Fig 3 for rat islets statically incubated in medium containing 3.3 mmol/L glucose and then shifted to a glucose concentration of 16.7 mmol/L, there was an increase in insulin output by nearly 20-fold ($P < .001$). Moreover, these levels of insulin release were comparable in either the absence or presence of (1:700) NRS (0.51 ± 0.08 *v* 0.45 ± 0.05 ng/islet/60 min at 3.3 mmol/L; 8.64 ± 0.53 *v* 8.95 ± 0.33 ng/islet/60 min at 16.7 mmol/L). By contrast, when ACTH-As was included in the medium at the same dilution, a sharp decrease in insulin output, amounting to some 25% of the control value, occurred at the higher level of glucose (6.48 ± 0.48 ng/islet/60 min, $P < .001$), thus indicating a markedly significant inhibition of insulin secretion following reduction of the effective concentration of endogenous ACTH-LP in situ by immunoneutralization. However, no such clear-cut

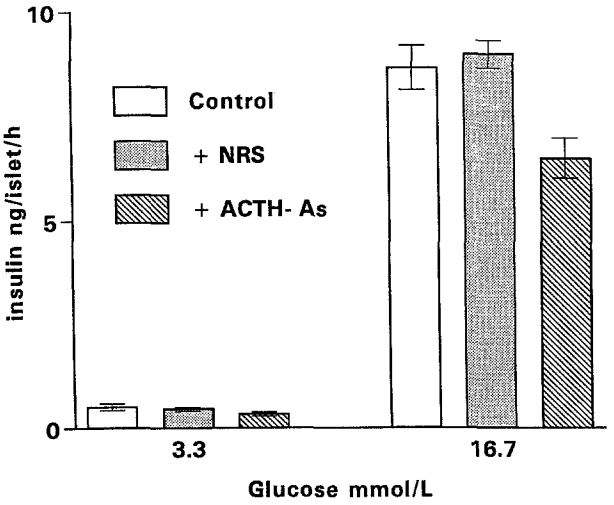

Fig 3. Effect of ACTH immunoneutralization in situ on rat islet glucose-induced insulin release. Supplementation of the islet medium with ACTH-As (final dilution, 1:700) decreased insulin release in the presence of low (3.3 mmol/L) glucose by only a slight and insignificant amount. However, this diminution became highly significant at a glucose concentration of 16.7 mmol/L ($P < .001$). By contrast, addition of NRS (final dilution, 1:700) did not produce any significant effect at either low or high concentrations of glucose. Each bar represents the mean ± SEM of 10 determinations.

effect on insulin secretion was seen upon exposure to ACTH-As at the lower glucose concentration, with the marginal decrement in those already-low release values under these conditions not attaining statistical significance (0.33 ± 0.05 ng/islet/60 min, $.05 < P < .07$, NS).

Insulin Release in the Presence of Glucose and CIP

When the effect of CIP was tested in a similar fashion (Fig 4), the normal rate of insulin release was not significantly affected by the peptide in the presence of 3.3 mmol/L glucose (0.29 ± 0.02 *v* 0.24 ± 0.03 ng/islet/60 min,

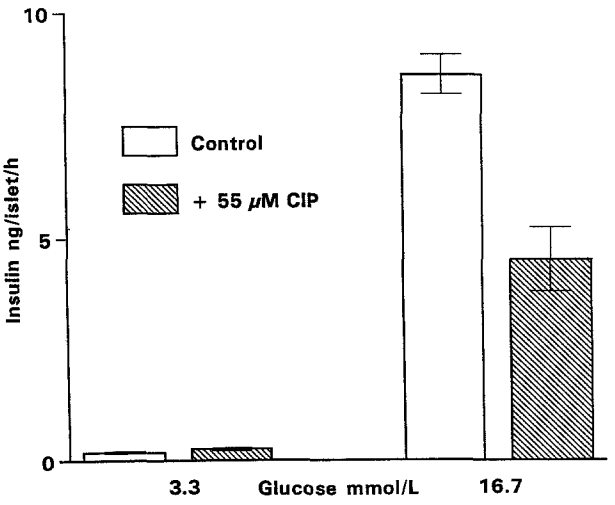

Fig 4. Effect of CIP on glucose-induced insulin release. Addition of 55 μmol/L CIP did not modify insulin secretion in the presence of 3.3 mmol/L glucose, but decreased the output significantly in 16.7 mmol/L glucose ($P < .001$). Each bar represents the mean ± SEM of 15 determinations.

respectively). However, CIP did substantially blunt (by ~50%, $P < .001$) the marked enhancement in insulin output that was otherwise evoked by an increase in glucose concentration to 16.7 mmol/L (8.64 ± 0.44 *v* 4.53 ± 0.72 ng/islet/60 min, control *v* CIP, respectively).

DISCUSSION

These results corroborate our previous report that ACTH-LP are secreted by isolated pancreatic islets in the static incubation procedure, and that the rate of release is altered by changes in glucose concentration.[13,14] Such differences in ACTH-LP output in response to medium glucose levels would suggest a modulated rather than a constitutive production of these peptides. Consistent with the previous findings of other[8-11,12] and our own results,[13,14] those more recent studies also showed that added ACTH can potentiate the secretion of insulin,[8-11,13,14] and that this capacity of the former as an insulin secretagogue also depends on the concentration of glucose in the incubation medium.[12] This present study now extends those observations to provide the first experimental evidence that these endogenously generated ACTH-LP are in fact required for a maximum output of insulin from rat islets in response to glucose-loading. The addition to islet incubation medium of either one of two factors that would be expected to interfere with ACTH action—a specific polyclonal antiserum raised against the full-length molecule (ACTH-As) and a potent ACTH receptor–blocking peptide (CIP)—significantly depressed insulin liberation in the presence of elevated glucose levels (Figs 3 and 4, respectively). That the inhibition of each of these two agents was seen only in the presence of high medium glucose (16.7 mmol/L, 300 mg/dL) would argue that the induction of insulin output by that sugar rather than its constitutive secretion under nonloading conditions (ie, 3.3 mmol/L, ~60 mg/dL) is partially mediated by ACTH; moreover, the strict specificity of both this antiserum and the peptide-blocking agent would further imply that among the family of ACTH-LP produced in response to elevated glucose,[13] the full-length ACTH per se—as opposed to its smaller posttranscriptionally processed cleavage products, such as α-MSH—is the species responsible for the observed secretagogue action. Although inhibition by ACTH-As was only about one half of that obtained with CIP, this weaker action on the part of the antiserum was no doubt a result of the excessive dilution we were forced to use for the experiment of Fig 3; moreover, we cannot be certain to what extent nascent ACTH-LP within the islets were effectively immunoneutralized under our experimental conditions. Thus, on the basis of the foregoing considerations, if one assumes that inhibition of CIP was both complete and quantitative under the conditions of our experiment (Fig 4), a good 50% of rat islet insulin release in response to elevated glucose would appear to be independent of the action of ACTH. The induction of this hormone by glucose-loading would thus be envisioned as a mechanism for fine-tuning the insulin response, especially in any situation where an independent downregulation of ACTH-LP production might be superimposed on a stimulation of insulin output by high glucose levels (ie, in the presence of glucocorticoids).

In this regard, we and others have provided evidence for the presence of POMC in rat islet cells.[3-7,13,24-27] Indeed, the high—molecular-weight transcript for this opioid precursor has been detected in the endocrine pancreas[2-4]; the level of this POMC-encoding message was diminished by dexamethasone in a manner antagonistic to the effect of glucose[4]; and the synthetic steroid inhibited glucose-induced release of insulin, as well.[28-30] Furthermore steady-state levels of ACTH-LP elaborated by rat islets, as measured by antibodies recognizing only the *N*-terminal portion of that molecule, were higher than those indicated by this present antiserum—raised against the midportion of ACTH—provided that the medium contained low glucose (3.3 mmol/L). This last observation implied that under such conditions, islets were producing greater amounts of the more extensively processed POMC species, such as α-MSH and other related peptides, than the full-length ACTH itself. However, when total ACTH-LP levels increased in response to induction by high medium glucose (16.7 mmol/L), this pattern became reversed, since the ACTH-LP species found to accumulate in this circumstance was $^{1\text{-}39}$ACTH, as opposed to its *N*-terminal cleavage products.[13] These observations thus indicated not only that the POMC of rat islet tissue was posttranscriptionally processed in a manner similar to that occurring in the intermediate lobe of the pituitary and in the central nervous system, but that both the production of this precursor peptide within the endocrine pancreas and the relative distribution of its subsequent cleavage products were subject to regulation by glucose concentration in the incubation medium.

The molar quantity of ACTH-LP secreted from isolated islets was only about 0.25% of the insulin output after a 20-minute perifusion with 16.7 mmol/L glucose (Fig 1). This suggests that, in contrast to the production of insulin, the amount of ACTH-LP released from the endocrine pancreas in vivo is, in all likelihood, insufficient to exert an endocrine effect on remote extraislet target tissues. Rather, the secretory rate of the former is consistent with the notion that ACTH-LP act locally within the islet in a paracrine fashion without even needing to enter the bloodstream. Such a form of intraislet compartmentalization would be expected to result in effective concentrations of these peptides that are well above the normal circulating levels in rats (ie, ~10 pmol/L).[31] Indeed, on the basis of measurements of ACTH-LP output rates per unit volume of islet interstitium, we have estimated that localized concentrations of these peptides in and around pancreatic B cells would be well within the range necessary for a facile stimulation of insulin secretion (~46 pmol/L[13]; Fig 2).

These considerations raise a question as to what levels of exogenously added ACTH itself would need to be present in an incubation medium to see an insulin-secretagogue effect with intact islets. Although others have already observed stimulations of insulin release under such conditions,[8-12] the concentrations they used were much higher than those we found to be effective in either our previously published study (71 pmol/L at 3.3 mmol/L glucose)[13] or the experiment of Fig 2 (ie, 55 pmol/L at 16.7 mmol/L glucose). However, even these values are severalfold above what would be deemed physiologic levels with respect to ACTH's classic endocrine functions (ie, ~10 pmol/L). However, if we assume that ACTH-LP act in a paracrine fashion within pancreatic islets, such experimental doses of $^{1\text{-}39}$ACTH may well be within the physiologic range for the endogenous insulinogenic action of that peptide on its target B cells. Finally, we should also consider that exogenously introduced ACTH may not fully penetrate into the islet core, at least within the time frame of our experiments, but that those molecules that did in fact reach the required site of action would be expected to interact additively in situ with any nascent ACTH-LP generated under the influence of glucose.

On the basis of the present studies and our previous investigations,[13,14] we therefore propose that ACTH-LP generated endogenously within rat islets mediate, at least in part, the induction of insulin secretion by glucose. This effect may, in turn, reflect the increases in cytosolic concentrations of cyclic 3′,5′-adenosine monophosphate and Ca^{2+} that have been reported to occur within pancreatic B cells under the influence of these POMC-related peptides.[14,15]

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REFERENCES

1. Mains RE, Eipper BA: The tissue-specific processing of pro-ACTH/endorphin. TEM 1:388-394, 1990

2. Zuhlke H, Jess G, Rosolski T, et al: Characterization of transcriptional and translational products of B-endorphin in isolated islets of Langerhans. Diabetologia 31:562A, 1988 (abstr)

3. Hummel A, Lendeckel U, Hahn V, et al: Presence and regulation of a truncated proopiomelanocortin gene transcript in rat pancreatic islets. Biol Chem Hoppe-Seyler 373:1039-1044, 1992

4. Zuhlke H, Lendeckel U, Zinke A, et al: Regulation of expression of proopiomelanocortin gene in isolated islets of Langerhans. Diabetologia 32:560A, 1989 (abstr)

5. Ehrenreich H, Goebel FD: The role of opioids in the endocrine function of the pancreas. Diabetes Res 3:59-66, 1986

6. Knudtzon J: Effects of proopiomelanocortin-derived peptides on plasma levels of glucagon, insulin, and glucose. Horm Metab Res 18:579-583, 1986

7. Marshall JB, Kapcala LP, Manning LD, et al: Effect of corticotropin-like intermediate lobe peptide on pancreatic exocrine function in isolated rat pancreatic lobules. J Clin Invest 74:1885-1889, 1984

8. Curry DL, Bennett LL: Dynamics of insulin release by perfused rat pancreases: Effects of hypophysectomy, growth hormone, adrenocorticotropic hormone, and hydrocortisone. Endocrinology 93:602-609, 1973

9. Genuth S, Lebovitz HE: Stimulation of insulin release by corticotropin. Endocrinology 76:1093-1099, 1965

10. Levobitz HE, Pooler K: ACTH-mediated insulin secretion: Effect of aminophylline. Endocrinology 81:558-564, 1967

11. Sussman KE, Vaughan GD: Insulin release after ACTH, glucagon, and adenosine-3′5′-phosphate (cyclic AMP) in the perfused isolated rat pancreas. Diabetes 16:449-454, 1967

12. Malaisse WJ, Malaisse-Lagae F, Mayhew D: A possible role

for the adenylcyclase system in insulin secretion. J Clin Invest 46:1724-1734, 1967

13. Borelli MI, Morano MI, Estivariz FE, et al: Glucose-induced secretion of ACTH-like products by rat pancreatic islets. Arch Int Physiol Biochim Biophys 102:17-20, 1992

14. Gagliardino JJ, Borelli MI, Boschero AC, et al: Modulatory mechanism of ACTH on insulin secretion: Effect on cytosolic Ca^{2+}, membrane potential, and Ca^{2+}-ATPase activity. Arch Physiol Biochim 103:73-78, 1995

15. Gagliardino JJ, Rossi JPFC: Ca^{2+}-ATPase in pancreatic islets: Its possible role in the regulation of insulin secretion. Diabetes Metab Rev 10:1-17, 1994

16. Lacy PE, Kostianovsky M: Method for the isolation of intact islets of Langerhans from rat pancreas. Diabetes 16:35-39, 1967

17. Kanatsuna T, Lernmark A, Rubenstein AH, et al: Block in insulin release from column-perifused pancreatic B-cells induced by islet cell surface antibodies and complement. Diabetes 30:231-234, 1981

18. Diaz GB, Paladini AA, Garcia ME, et al: Changes induced by hypothyroidism in insulin secretion and in the properties of islet plasma membranes. Arch Int Physiol Biochim Biophys 101:263-269, 1993

19. Morano MI, Estivariz FE: Chromatographic characteristics of proopiomelanocortin-derived peptides from the rat transplantable tumor 73315 a. J Endocrinol 112:417-425, 1987

20. Fenger M: Alpha-melanocyte-stimulating-hormone precursors in the pig pituitary. Biochem J 235:715-722, 1986

21. Linde S, Hansen B, Lernmark A: Preparation of stable radioiodinated polypeptide hormone and proteins using polyacrylamide gel electrophoresis. Anal Biochem 107:165-171, 1980

22. Herbert V, Lau KS, Gottlieb CW, et al: Coated-charcoal immunoassay of insulin. J Clin Endocrinol Metab 25:1375-1384, 1965

23. Gagliardino JJ, Nierle C, Pfeiffer EF: The effect of serotonin on in vitro insulin secretion and biosynthesis in mice. Diabetologia 10:411-414, 1974

24. Niendorf WR, Zuhlke H: Biosynthesis of β-endorphin in pancreatic islets of neonatal Wistar rats. Biomed Biochim Acta 442:51-54, 1985

25. Bruni JF, Watkins WB, Yen SSC: β-Endorphin in the human pancreas. J Clin Endocrinol Metab 49:649-651, 1979

26. Grube D, Voight KH, Weber E: Pancreatic glucagon cells contain endorphin-like immunoreactivity. Histochemistry 59:75-79, 1976

27. Sanchez Franco F, Patel YC, Reichlin S: Immunoreactive adrenocorticotropin in the gastrointestinal tract and pancreatic islets of the rat. Endocrinology 108:834-840, 1981

28. Barseghian G, Levine B: Effect of corticosterone on insulin and glucagon secretion of the isolated perfused rat pancreas. Endocrinology 106:547-554, 1980

29. Billaudel B, Sutter BC: Direct effect of corticosterone upon insulin secretion studied by three different techniques. Horm Metab Res 11:555-569, 1979

30. Gronda CM, Diaz GB, Rossi JPFC, et al: Correlation between Ca^{2+}-ATPase activity of rat islet cells and insulin secretion. J Endocrinol 134:221-225, 1992

31. Estivariz FE, Morano MI, Carino M, et al: Adrenal regeneration in the rat is mediated by mitogenic *N*-terminal proopiomelanocortin peptides generated by changes in precursor processing in the anterior pituitary. J Endocrinol 116:207-216, 1988