Direct Modulation of Pancreatic CCK Receptors and Enzyme Secretion by Insulin in Isolated Pancreatic Acini from Diabetic Rats

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SUMMARY

The effect of insulin on pancreatic enzyme secretion was studied in vitro by using isolated pancreatic acini prepared from rats rendered diabetic with streptozotocin. Preincubation of acini with insulin increased the maximal release of amylase and ribonuclease in response to CCK_8 but not to carbamylcholine, a secretagogue acting via a different receptor. Insulin by itself had no effect on enzyme release. The effect of insulin was time dependent and increased up to 2 h, the longest time studied. An effect was observed at 1 nM insulin and the maximal effect was observed at 100 nM. The properties of CCK receptors on the acini were studied using biologically active ^{125}I-BH-CCK_{33}. Insulin pretreatment decreased both the affinity of the high-affinity CCK receptors and the capacity of the low-affinity CCK receptors. Within the framework of the current model, in which occupancy of high-affinity CCK receptors stimulates and low-affinity receptors inhibits amylase release, the change in receptors induced by insulin could account for the altered enzyme secretion. Thus, insulin appears to have a direct effect to regulate CCK receptors and CCK-induced secretion in isolated pancreatic acini. DIABETES *32*:241–246, March 1983.

Exocrine pancreatic function in both man and animals is now known to be influenced by insulin.[1–9] In concert with this finding, a portal capillary network has been demonstrated to exist between the islets of Langerhans and the acini such that the islet hormones will reach the acinar tissue in high concentrations before they enter the systemic circulation.[10,11] In rats, insulin in vivo is known to alter the concentration of pancreatic digestive enzymes through an effect on messenger RNA levels.[8,12] We have recently shown that streptozotocin-induced diabetes also leads to altered maximal rates of secretion that parallel the changes in the enzyme content of acinar cells.[13,14] In addition, a selective decrease in sensitivity to CCK was observed that could be reversed by administration of insulin in vivo.[14] Since insulin is known to have direct in vitro effects to regulate protein synthesis in isolated acini prepared from diabetic rats,[15] we studied the effects of insulin in vitro on the release of digestive enzymes induced by CCK and carbamylcholine.

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MATERIALS AND METHODS

Materials. The following chemicals were purchased: streptozotocin, soybean trypsin inhibitor (type 1-S), carbamylcholine chloride (carbachol), ribonuclease A (type X-A), amylase (type 1-A), and calf thymus DNA from Sigma Chemical Co. (St. Louis, Missouri); chromatographically purified collagenase and STATZYME glucose kit from Worthington Biochemicals (Freehold, New Jersey); minimal Eagle's medium amino acid supplement from GIBCO (Grand Island, New York); bovine serum albumin (fraction V) from Miles Laboratories (Elkhart, Indiana) and from Reheis (Chicago, Illinois); porcine insulin from Elanco Products (Indianapolis, Indiana); ribonucleic acid (grade A) from Calbiochem (La Jolla, California); 3,5-diaminobenzoic acid from Aldrich Chemical Co. (Milwaukee, Wisconsin); N-succinimidyl 3-(4-hydroxy, 5-[^{125}I]iodophenyl) propionate (^{125}I-Bolton Hunter reagent) from Amersham (Arlington Heights, Illinois). Pure natural porcine cholecystokinin (CCK_{33}) was obtained from the Gastrointestinal Hormone Laboratory, Karolinska Institute (Stockholm, Sweden). Cholecystokinin octapeptide (CCK_8) was a gift from Dr. Miguel Ondetti of the Squibb Institute for Medical Research (Princeton, New Jersey).

CCK_{33} was radioiodinated with full retention of biologic activity by conjugation with ^{125}I-BH-reagent to a specific activity of 200–300 μCi/μg, as described previously.[16]

Male Sprague-Dawley rats (Simonsen Laboratories, Gilroy, California), 120–140 g, were fasted 24 h and injected

via the tail vein with streptozotocin (75 mg/kg) freshly dissolved in 0.1 M sodium citrate buffer (pH 4.3). The animals were then fed ad libitum with Purina Rat Chow and kept at 23°C in a 12-h-light-dark–cycled room. The rats that gained weight at the rate of 0–20 g/wk were selected for study and were used 15–19 days after the injection.[14] All animals were decapitated after a blow to the head between 9:30 and 10:30 a.m. to exclude any circadian variation. Blood glucose levels determined in the morning at the time of death averaged 571.1 ± 14.6 mg/dl (mean ± SE, N = 62).

Preparation of isolated pancreatic acini. Isolated pancreatic acini from diabetic rats were prepared by the methods reported previously.[14,17] Pancreata (1.0–1.1 g) obtained from two rats were injected with 5 ml Krebs-Henseleit bicarbonate buffer (KHB) with 95% O_2 and 5% CO_2, pH 7.4, containing 0.1 mM Ca^{2+}, 11.1 mM glucose, 2 mg/ml bovine serum albumin, 0.1 mg/ml soybean trypsin inhibitor, 40–45 U/ml purified collagenase, and minimal Eagle's medium amino acid supplement.[14] The injected pancreatic tissue was then incubated in a 25-ml polycarbonate Erlenmeyer flask at 37°C shaking 120 times/min. After 10 min, the dissociation medium was replaced with 5 ml fresh medium and the incubation continued for another 40 min. The pancreatic tissue was then mechanically dissociated by forceful pipetting through plastic pipettes and acini were isolated by filtration through 150-μm mesh nylon cloth, and centrifugation through KHB containing 4% bovine serum albumin. Acini were washed twice with the same buffer and then once with N-2-hydroxy ethylpiperazine-N′-2′-ethanesulfonic acid (HEPES) buffered Ringer (HR). HR[14] contained 10 mM HEPES, 1.28 mM Ca^{2+}, 11.1 mM glucose, minimal Eagle's medium amino acid supplement, 0.1 mg/ml soybean trypsin inhibitor, and 0.5% bovine serum albumin of low insulin-like activity. This HR was equilibrated with 100% O_2 and adjusted to pH 7.4 at 37°C.

In all experiments, acini were allowed to recover in HR for 60 min at a density of 1.2–1.5 mg acinar protein/ml in plastic flasks that were shaken at 60 cycles/min in a 37°C water bath.

Enzyme secretion. After recovery, acini were centrifuged at 50 × *g* for 2 min and resuspended in fresh HR at a density of 0.8–1.0 mg protein/ml. Acini were then preincubated for a further 2 h at 37°C with shaking at 60 times/min in the presence or absence of insulin. After this treatment, acini were centrifuged and resuspended in fresh HR at a density of 0.3–0.4 mg protein/ml. Two-milliliter aliquots were distributed into 25-ml polycarbonate Erlenmeyer flasks and secretagogues added in the presence or absence of insulin.

Enzyme release in response to various concentrations of cholecystokinin octapeptide (CCK_8) or carbamylcholine was determined using the procedure reported previously.[17] At the beginning of each incubation, 1 ml of acinar suspension was centrifuged at 10,000 × *g* for 20 s in an Eppendorf microcentrifuge. The enzyme activity in the supernatant was subtracted from the values obtained similarly after incubation to determine the enzyme release during the incubation. The acinar pellets from the initial samples were rinsed with 0.9% NaCl and recentrifuged. One milliliter of water was then added to each cell pellet and sonicated with a probe-type sonicator. Amylase and ribonuclease activities and protein and DNA concentrations were determined. Enzyme release was calculated as the percentage of the total content of the enzyme in the acinar pellet that was released.

In studies of the time course of insulin's action on CCK_8-stimulated amylase release, isolated acini were preincubated for 2 h at 37°C. At the indicated times before termination of this period, insulin was added to give a final concentration of 100 nM. At the end of the pretreatment, all acini were simultaneously centrifuged and resuspended in HR with or without insulin. Amylase release in response to 1 nM CCK_8 was then determined during a subsequent 30-min incubation at 37°C.

Binding studies. CCK binding to its receptors was determined as previously reported.[18] After a 2-h preincubation in the presence or absence of 100 nM insulin, isolated acini were resuspended at a protein concentration of 0.15 to 0.3 mg/ml in aforementioned HR that was enriched with 0.5 mg/ml bacitracin to reduce degradation of the ^{125}I-BH-CCK.[19] Four-milliliter aliquots were distributed in plastic tissue culture flasks, ^{125}I-BH-CCK_{33} (10 pM as determined by bioassay) plus various concentrations of unlabeled CCK_{33} were added to this suspension, and incubation was carried out for 60 min in a 37°C water bath. Triplicate 1-ml samples were removed from each flask and centrifuged at 300 × *g* for 2 min at 4°C. The pellets thus obtained were washed twice with saline at 4°C and the radioactivity associated with the washed acinar pellet was determined in a gamma scintillation counter. Total radioactivity in the medium from each flask was also determined. Nonspecific binding was determined by incubating acini with labeled CCK in the presence of an excess of CCK_8 (500 nM). Competitive inhibition of specific ^{125}I-BH-CCK binding by varying concentrations of unlabeled CCK_{33} was analyzed by fitting plots of bound hormone versus free hormone with a nonlinear least-squares computer program that analyzed the data in terms of one nonsaturable and two saturable components.[19,20] The affinity and capacity of each binding site were calculated and the data for the saturable components were replotted according to the method of Scatchard.[21]

Degradation of labeled hormone during the binding study was determined by adding 100 μl of supernatant from the acinar suspension to 0.5 ml of 20% trichloroacetic acid at 4°C followed in rapid succession by 0.4 ml of HR to provide carrier protein. The radioactivity in the supernatant and precipitate, obtained after centrifugation at 1000 g for 10 min at 4°C, was then counted.

Assays. Amylase activity was determined by the method of Jung[22] using procion yellow starch as substrate. Ribonuclease activity was measured by the modified method of Anfinsen et al.[23] using yeast ribonucleic acid as substrate. Protein was assayed by the method of Lowry et al.[24] using bovine serum albumin as a standard. DNA was measured fluorometrically by the reaction between 3,5-diaminobenzoic acid and deoxyribose sugar with calf thymus DNA used as a standard.[25] Serum glucose concentrations were measured by the hexokinase method[26] with the Worthington STATZYME kit.

RESULTS

Incubation with insulin of pancreatic acini prepared from diabetic rats increased the subsequent release of amylase and ribonuclease induced by CCK_8 (Figure 1). Insulin had no effect on basal and enzyme release stimulated by 300 pM or lower concentrations of CCK_8 but increased the enzyme release in response to 1 nM and higher concentrations

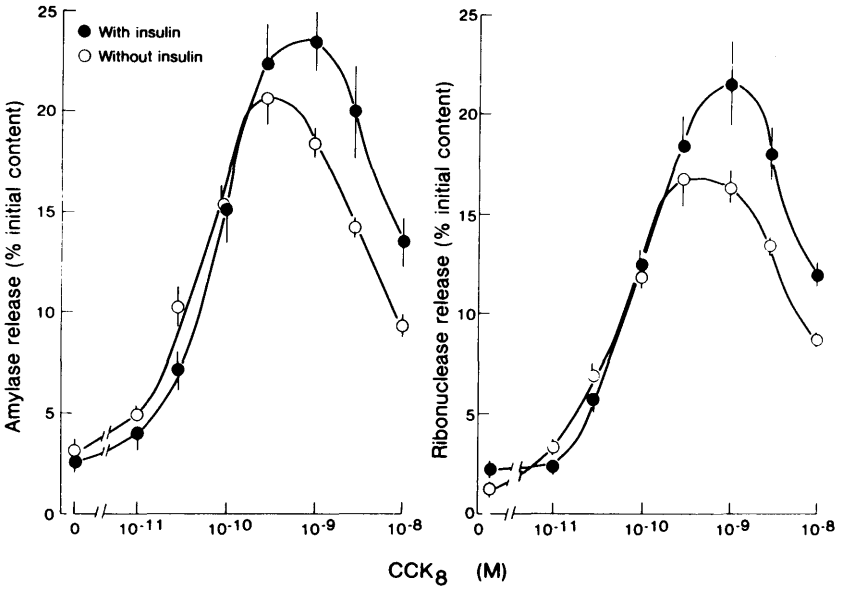

FIGURE 1. Dose response of CCK_8-induced amylase and ribonuclease release in the presence and absence of insulin. Pancreatic acini prepared from diabetic rats were incubated 2 h with or without 100 nM insulin followed by a 30-min incubation in similar fresh medium containing the specified concentration of CCK_8. All values are the mean ± SE of six experiments.

of CCK_8. As a result of this effect of insulin, the CCK dose-response curve for enzyme release shifted toward higher concentration; maximal secretion from diabetic rat acini incubated with insulin occurred at 1 nM while that without insulin occurred at 300 pM CCK_8.

The ability of insulin to potentiate the response to CCK_8 was dose dependent (Figure 2). A detectable effect was observed at 1 nM and the maximal effect was observed at 100 nM insulin. The effect of insulin was time dependent and increased up to 2 h (Figure 3). Experiments with a longer exposure were not undertaken since the viability of the acini decreased with longer incubation. Subsequent experiments were therefore carried out with acini incubated for 2 h in the presence or absence of 100 nM insulin. Insulin by itself had no significant effect on enzyme secretion.

Since cholinergic agonists act on a different receptor than CCK, but both appear to activate a common intracellular mechanism, the interaction between insulin and carbamylcholine was studied with respect to amylase release (Figure 4). In contrast to CCK_8, the maximal amylase release in response to carbamylcholine from acini treated with 100 nM insulin was the same as that from control acini. The sensitivity to carbamylcholine was slightly decreased in acini preincubated with insulin, manifested by about a twofold shift in the dose-response curve for amylase.

Since the potentiation of secretion was observed for CCK

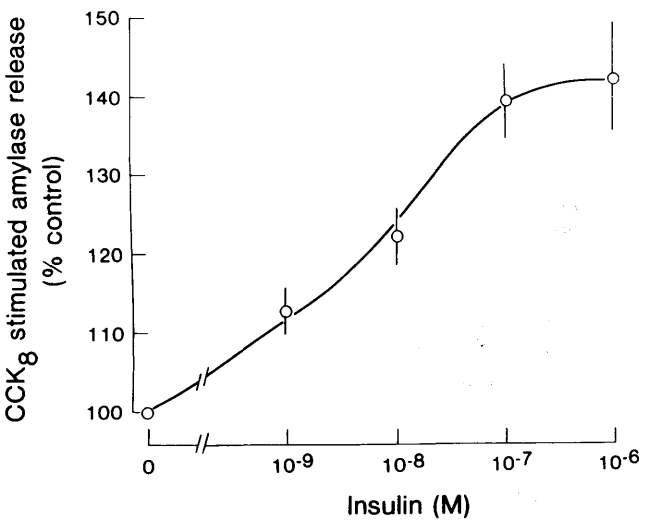

FIGURE 2. Concentration dependence for the potentiation by insulin of amylase release in response to CCK_8. Acini prepared from diabetic rats were incubated 2 h with the specific concentration of insulin and then incubated 30 min with 1 nM CCK_8. All values are the mean ± SE of four experiments.

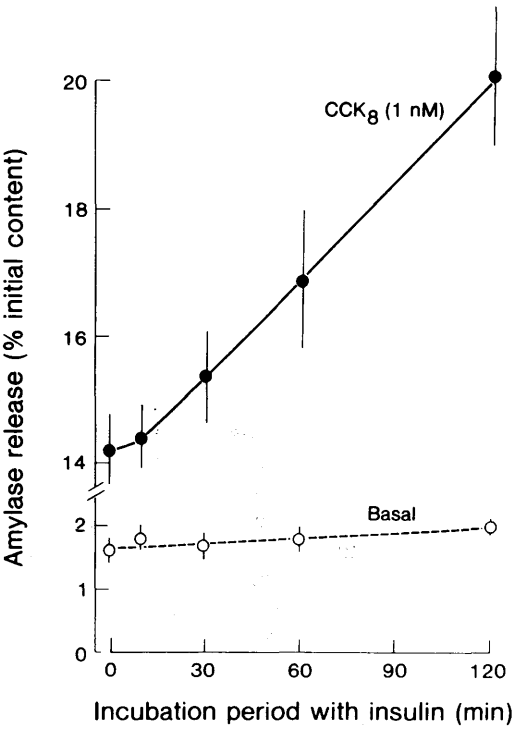

FIGURE 3. Effect of the duration of incubation with insulin on subsequent CCK_8-stimulated amylase release. Acini were incubated 2 h with insulin present for the specified time and then incubated 30 min with or without 1 nM CCK_8. All values are the mean ± SE of four experiments. Note the break in scale between the basal and CCK_8-stimulated amylase release.

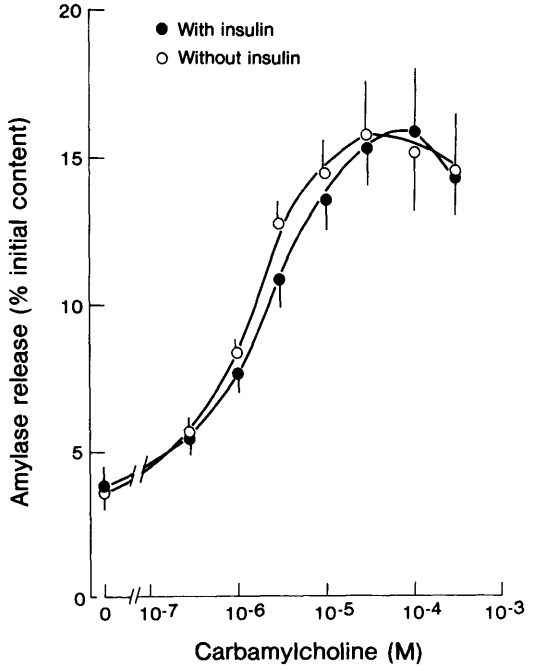

FIGURE 4. Effect of insulin on carbamylcholine-stimulated amylase release. Pancreatic acini were incubated 2 h with or without 100 nM insulin followed by a 30-min incubation in similar fresh medium containing the specified concentration of carbamylcholine. All values are the mean ± SE of four experiments.

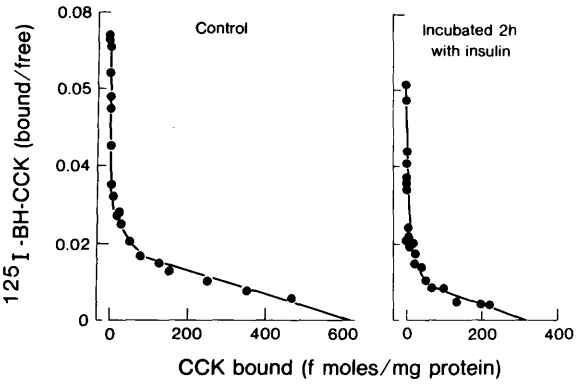

FIGURE 5. Representative Scatchard plots of ^{125}I-BH-CCK binding to acini incubated 2 h with or without 100 nM insulin.

but not carbamylcholine it seemed likely that insulin might be affecting the CCK receptor or an early step in the mechanism by which CCK activates enzyme secretion. To directly study the properties of the CCK receptor, the binding of ^{125}I-BH-CCK to the CCK receptor was studied. ^{125}I-BH-CCK binding to diabetic acini was maximal after 60 min.[27] During this incubation period about 15% of the ligand was degraded regardless of the presence or absence of insulin as measured by the appearance of trichloroacetic acid-soluble radioactivity. Under these conditions, ^{125}I-BH-CCK binding to diabetic rat acini treated with or without insulin was competitively inhibited by increasing concentrations of unlabeled CCK_{33}. The affinity and capacity of binding at steady state (60 min) were determined by computer analysis and the saturable components plotted as bound/free versus bound CCK (Figure 5). These Scatchard plots of acini with or without insulin were curvilinear and compatible with two classes of binding sites. Insulin treatment decreased both the affinity of the high-affinity binding site and the capacity of the low-affinity site (Table 1). By contrast, the capacity of the high-affinity sites and affinity of the low-affinity sites were not altered by insulin.

DISCUSSION

Pancreatic acinar cell function is regulated by a number of hormones and neurotransmitters. Secretion of digestive enzymes is primarily stimulated by the hormone CCK, which is released from the small intestine, and by the neurotransmitter acetylcholine, which is released from vagal nerve endings. Insulin released from the pancreatic islet B-cell is known to regulate the dietary adaptation of the enzyme content in the pancreas and potentiate some of the actions of CCK and acetylcholine.[28]

Insulin by itself has been found not to alter pancreatic enzyme secretion from isolated acini prepared from normal and diabetic rat pancreas (present work, unpublished data) or from the perfused rat pancreas.[28] The recent report that suggested that insulin was as potent as and mimicked all the effects of CCK in the partially digested rat pancreas[29] cannot be explained unless the insulin used in that experiment was contaminated with CCK. Insulin, however, does potentiate the response of isolated pancreatic acini to high concentrations of CCK as shown here and to the CCK analogue, caerulein, as reported previously.[30] Insulin has also been shown to potentiate the exocrine pancreatic response to both CCK and carbamylcholine in the perfused rat pancreas.[28] In the perfused pancreas, however, the effect of insulin was rapid, occurring within 10 min. Since in the perfused pancreas enzyme secretion occurs together with pancreatic juice flow, it is possible that insulin enhances a step such as fluid secretion that is no longer a requirement for secretion by isolated acini.

The present study was carried out using acini prepared from diabetic rats since these acini are more sensitive to insulin than acini from normal rats. This increased sensitivity of diabetic rat acini to exogenous insulin may likely be due to the much lower insulin contamination in the incubation medium from endogenous islet B-cells.[15] Isolated pancreatic acini from normal and diabetic rats have been demonstrated to contain insulin receptors,[31] and insulin is known to stimulate protein synthesis[15] and to a small extent glucose transport (unpublished data) in these acini. Of interest, the potentiation of CCK-induced amylase release occurred over a similar insulin concentration range (1–100 nM), as did the stimulation of leucine incorporation into protein.[15] These concentrations of insulin are higher than those normally used to stimulate protein synthesis and glucose metabolism in fat cells, liver, and muscle. The pancreatic acinar cells are, however, normally exposed to concentrations of insulin 50–100-fold greater than those found in peripheral blood.

Previous comparison of secretion by acini from normal and diabetic rats indicated a threefold reduction in sensitivity to CCK in diabetics that could be reversed by administration of insulin in vivo.[14,27] The present study indicates that the direct or in vitro effects of insulin on exocrine pancreas are different from those observed after administration of insulin

TABLE 1
CCK binding parameters of isolated acini prepared from diabetic rats in the presence and absence of insulin

	Diabetic acini incubated 2 h without insulin (N = 10)	Diabetic acini incubated 2 h with 100 nM insulin (N = 7)	P
High-affinity site			
Kd (pM)	68.6 ± 9.9	105.7 ± 10.5	< 0.01
Capacity (fmol/mg protein)	4.8 ± 0.7	4.2 ± 0.7	NS
Low-affinity site			
Kd (nM)	25.4 ± 5.7	24.4 ± 4.7	NS
Capacity (fmol/mg protein)	649.8 ± 127.6	326.8 ± 43.2	< 0.01

in vivo.[14] However, it strongly indicates that the enhancement of secretion is accompanied by and probably due to an effect of insulin on CCK receptors. First, the effect was specific for CCK since there was no enhancement of secretion by carbamylcholine; these two secretagogues are known to act on distinct receptors, but to activate a common intracellular mechanism involving the mobilization of Ca^{2+}. Second, insulin had direct effects on CCK receptors; both the affinity of the high-affinity sites and the capacity of low-affinity sites decreased by incubation with insulin.

CCK binding to pancreatic acini of both rats and mice yields curvilinear Scatchard plots that are most easily interpreted as indicating two classes of binding sites.[18,19] Regulation of amylase release by these receptors is complex since the amylase release dose-response curve is biphasic with low concentrations of CCK stimulating, but with high concentrations inhibiting, enzyme release. We have proposed that the biphasic dose-response curve can be explained by a model in which occupancy of the high-affinity CCK receptors stimulates amylase release and occupancy of low-affinity receptors inhibits release.[19] In this model at any concentration of CCK both high- and low-affinity receptors are occupied and the resulting amylase release depends on the interaction between the two classes of receptors. The present observation that insulin leads to a decreased number of the low-affinity CCK receptors could explain how high concentrations of CCK could induce higher amounts of amylase release. Further work is clearly needed to understand the mechanism by which occupancy of CCK receptors leads to the stimulation of amylase release and how this mechanism is altered by insulin.

In conclusion, the present work supports the concept that insulin acts as a positive modulator of CCK action on pancreatic acini, including stimulation of digestive enzyme release, and suggests that one mechanism is through alteration of the properties of the CCK receptor.

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REFERENCES

[1] Chey, W. Y., Shay, H., and Shuman, C. R.: External pancreatic secretion in diabetes mellitus. Ann. Intern. Med. *59*:812–21, 1963.

[2] Vacca, J. B., Henke, W. J., and Knight, W. A., Jr.: The exocrine pancreas in diabetes mellitus. Ann. Intern. Med. *61*:242–47, 1964.

[3] Domschke, W., Tympner, F., Domschke, S., and Demling, L.: Exocrine pancreatic function in juvenile diabetes. Am. J. Digest. Dis. *20*:309–12, 1975.

[4] Frier, B. M., Faber, O. K., Binder, C., and Elliott, H. L.: The effect of residual insulin secretion on exocrine pancreatic function in juvenile-onset diabetes melllitus. Diabetologia *14*:301–304, 1978.

[5] Ben Abdeljlil, A., Palla, J. C., and Desneulle, P.: Effect of insulin on pancreatic amylase and chymotrypsinogen. Biochem. Biophys. Res. Commun. *18*:71–75, 1965.

[6] Snook, J. T.: Effect of diet, adrenalectomy, diabetes, and actinomycin D on exocrine pancreas. Am. J. Physiol. *215*:1329–33, 1968.

[7] Christophe, J., Camus, J., Deschodt-Lanckman, M., Rathe, J., Robberecht, P., Vandermeers-Piret, M. C., and Vandermeers, A.: Factors regulating biosynthesis, intracellular transport and secretion of amylase and lipase in the rat exocrine pancreas. Horm. Metab. Res. *3*:393–409, 1971.

[8] Söling, H. D., and Unger, K. O.: The role of insulin in the regulation of α-amylase synthesis in the rat pancreas. Eur. J. Clin. Invest. *2*:199–212, 1972.

[9] Adler, G., and Kern, H. F.: Regulation of exocrine pancreatic secretory process by insulin in vivo. Horm. Metab. Res. *7*:290–96, 1975.

[10] Henderson, J. R., and Daniel, P. M.: A comparative study of the portal vessels connecting the endocrine and exocrine pancreas, with a discussion of some functional implications. Q. J. Exp. Physiol. *64*:267–75, 1979.

[11] Lifson, N., Kramlinger, K. G., Mayrand, R. R., and Lender, E. J.: Blood flow to the rabbit pancreas with special reference to the islets of Langerhans. Gastroenterology *79*:466–73, 1980.

[12] Korc, M., Owerbach, D., Quinto, C., and Rutter, W. J.: Pancreatic islet-acinar cell interaction: amylase messenger RNA levels are determined by insulin. Science *213*:351–53, 1981.

[13] Otsuki, M., and Williams, J. A.: Effects of experimental diabetes on pancreatic enzyme content and secretion in vitro. Clin. Res. *29*:86A, 1981.

[14] Otsuki, M., and Williams, J. A.: Effect of diabetes mellitus on the regulation of enzyme secretion by isolated rat pancreatic acini. J. Clin. Invest. *70*:148–56, 1982.

[15] Korc, M., Iwamoto, Y., Sankaran, H., Williams, J. A., and Goldfine, I. D.: Insulin action in pancreatic acini from streptozotocin-treated rats. I. Stimulation of protein synthesis. Am. J. Physiol. *240* (Gastrointest. Liver Physiol. 3):G56–62, 1981.

[16] Sankaran, H., Deveney, C. W., Goldfine, I. D., and Williams, J. A.: Preparation of biologically active radioiodinated cholecystokinin for receptor assay and radioimmunoassay. J. Biol. Chem. *254*:9349–51, 1979.

[17] Williams, J. A., Korc, M., and Dormer, R. L.: Action of secretagogues on a new preparation of functionally intact, isolated pancreatic acini. Am. J. Physiol. *235* (Endocrinol. Metab. Gastrointest. Physiol. 4):E517–24, 1978.

[18] Sankaran, H., Goldfine, I. D., Deveney, C. W., Wong, K. Y., and Williams, J. A.: Binding of cholecystokinin to high affinity receptors on isolated rat pancreatic acini. J. Biol. Chem. *255*:1849–53, 1980.

[19] Sankaran, H., Goldfine, I. D., Bailey, A. C., Licko, V., and Williams, J. A.: Relationship of CCK receptor binding to regulation of biological functions in pancreatic acini. Am. J. Physiol. *242*(Gastrointest. Liver Physiol. *5*):G250–57, 1982.

[20] Dixon, W. J., Ed.: BMD Biomedical Computer Programs. Berkeley, Cal., University of California, 1974.

[21] Scatchard, G.: The attractions of proteins for small molecules and ions. Ann. NY Acad. Sci. *51*:660–72, 1949.

[22] Jung, D. H.: Preparation and application of procion yellow starch for amylase assay. Clin. Chim. Acta *100*:7–11, 1980.

[23] Anfinsen, C. B., Redfield, R. R., Choate, W. L., Page, J., and Carroll, W. R.: Studies on the gross structure, cross-linkages, and terminal sequences in ribonuclease. J. Biol. Chem. *207*:201–10, 1954.

[24] Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J.: Protein measurement with Folin phenol reagent. J. Biol. Chem. *193*:265–75, 1951.

[25] Hinegardner, R. T.: An improved fluorometric assay for DNA. Anal. Biochem. *39*:197–201, 1971.

[26] Bondar, R. L. J., and Mead, D.: Evaluation of glucose-6-phosphate dehydrogenase from Leuconostoc mesenteroides in the hexokinase method for determining glucose in serum. Clin. Chem. *20*:586–90, 1974.

[27] Otsuki, M., and Williams, J. A.: Alteration of pancreatic cholecystokinin receptors and secretion by diabetes mellitus. Submitted for publication.

[28] Saito, A., Williams, J. A., and Kanno, T.: Potentiation of cholecysto-

kinin-induced exocrine secretion by both exogenous and endogenous insulin in isolated and perfused rat pancreata. J. Clin. Invest. *65*:777–82, 1980.

[29] Farese, R. V., Larson, R. E., and Sabir, M. A.: Insulin and its secretagogues activate Ca^{2+}-dependent phosphatidylinositol breakdown and amylase secretion in rat pancreas in vitro. Diabetes 30:396–401, 1981.

[30] Williams, J. A., Sankaran, H., Korc, M., and Goldfine, I. D.: Receptors for cholecystokinin and insulin in isolated pancreatic acini: hormonal control of secretion and metabolism. Fed. Proc. *40*:2497–502, 1981.

[31] Sankaran, H., Iwamoto, Y., Korc, M., Williams, J. A., and Goldfine, I. D.: Insulin action in pancreatic acini from streptozotocin-treated rats. II. Binding of ^{125}I-insulin to receptors. Am. J. Physiol. *240*(Gastrointest. Liver Physiol. *3*):G63–68, 1981.