Effect of Diabetes Mellitus on the Regulation of Enzyme Secretion by Isolated Rat Pancreatic Acini

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ABSTRACT The nature and mechanism of the pancreatic exocrine dysfunction in diabetes mellitus were evaluated in vitro using isolated pancreatic acini prepared from streptozotocin-induced diabetic rats. The content of amylase and ribonuclease in diabetic acini was ~0.5 and 50% of the normal content, respectively. Further, reduced amounts of both enzymes were secreted by diabetic acini in response to both cholecystokinin (CCK) and carbamylcholine. However, when enzyme secretion was normalized relative to initial acinar contents, both normal and diabetic acini released enzymes at a comparable maximal rate. The time course of the release of these enzymes, and newly synthesized protein were similar in both acini. In normal acini, the effect of CCK was maximal at a concentration of 100 pM; higher concentrations led to submaximal enzyme release. The dose-response curve in diabetic acini was similarly shaped, but shifted threefold towards higher concentration. The mobilization of cellular Ca^{2+} in response to CCK was also shifted. In contrast to these results with CCK, the dose-response curve to carbamylcholine was unaltered by diabetes. The observed effects were confirmed to be due to insulin deficiency and not due to direct toxic effect of streptozotocin on acinar cells or malnutrition. Streptozotocin had no acute effect on acini when measured 24 h after administration, and alloxan, another beta cell toxin, induced similar changes in acinar enzyme content and secretory response. Moreover, the administration of exogenous insulin to diabetic rats returned the content of pancreatic amylase and the secretory response to CCK towards normal. Starvation for 48 h, although inducing a significant weight loss, did not mimic the effects of diabetes. The present studies demonstrate two major abnormalities in pancreatic exocrine secretion in the diabetic rat: (*a*) the content of certain digestive enzymes is markedly altered, leading to an altered amount of zymogen secretion, (*b*) the sensitivity to CCK is selectively reduced, most likely related to a defect in receptor activated transmembrane signaling.

INTRODUCTION

Diabetes mellitus in man is often associated with exocrine pancreatic dysfunction, especially in patients with juvenile-onset (type 1) diabetes (1–5). These abnormalities include a reduced concentration of enzymes and bicarbonate in pancreatic juice after the intravenous injection of secretin and cholecystokinin (CCK).[1] Although a reduction in amylase output has been most frequently observed (1, 2), decreased secretion of trypsin and chymotrypsin have also been noted (3, 4). In general, the severity of these enzyme deficiencies is correlated with the duration of the disease (4). Along with these secretory abnormalities, a biosynthetic abnormality in the exocrine pancreas has been suggested by the finding of reduced pancreatic uptake of the amino acid analog selenomethionine (6, 7). In addition to these functional abnormalities of the exocrine pancreas in diabetes, pathological lesions in the pancreas have been reported including atrophy, arteriosclerosis, fibrosis, and fatty degeneration (8).

Because of the difficulty in studying human pancreatic tissue, the function of the exocrine pancreas has been studied in experimental animals with chemically induced diabetes mellitus. Induction of diabetes

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[1] *Abbreviations used in this paper*: CCK, cholecystokinin; CCK_8, cholecystokinin octapeptide; HR, Hepes-buffered Ringer's solution; KHB, Krebs-Henseleit bicarbonate buffer.

by alloxan or streptozotocin dramatically reduces pancreatic amylase content in rats; this reduction can be reversed by the in vivo administration of insulin (9–14). Although the pancreatic content of digestive enzymes can be readily determined in pancreas obtained from intact animals and related to secretion in vivo, other aspects of the secretory process, including intracellular transport of newly synthesized protein, quantitative study of the discharge of enzymes in response to secretagogues, and characterization of hormone receptors, can only be examined in detail with in vitro pancreatic preparations.

Regulation of pancreatic acinar cell function in vitro has been studied and quantitated most extensively in isolated pancreatic acini. This in vitro preparation is structurally and functionally similar to the acini of the intact pancreas, but is free of duct cells, islet cells, and connective tissue (15). Furthermore, in this experimental system, other variables such as the autonomic nervous system and alterations of blood flow to the exocrine pancreas are not present. Recently we reported that isolated acini can also be prepared from rats with diabetes induced by streptozotocin administration (16). In the present study, therefore, we used isolated pancreatic acini prepared from streptozotocin-induced diabetic rats to measure the content and secretion of two pancreatic digestive enzymes, amylase, and ribonuclease. Moreover, we investigated the effect of insulin treatment on both the content of pancreatic enzyme and the stimulation of amylase release in response to secretagogues.

METHODS

Materials. The following were purchased: streptozotocin, alloxan monohydrate, soybean trypsin inhibitor (type 1-S), hyaluronidase (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, MO; chromatographically purified collagenase, chymotrypsin, and Statzyme glucose kit from Worthington Biochemicals, Freehold, NJ; minimal Eagle's medium amino acid supplement from Gibco Laboratories, Grand Island Biological Co., Grand Island, NY; bovine serum albumin, fraction V, from Miles Laboratories, Elkhart, IN, and from Reheis, Chicago, IL; insulin (Lente) from E. R. Squibb & Sons, Inc., Princeton, NJ; 4,5-[^{3}H]leucine (57.1 Ci/mmol) and $^{45}CaCl_2$ (20 Ci/mmol) from New England Nuclear, Boston, MA; amylose azure and ribonucleic acid grade A, from Calbiochem-Behring Corp., La Jolla, CA; 3,5-diaminobenzoic acid from Aldrich Chemical Co., Milwaukee, WI. Cholecystokinin octapeptide (CCK_8) was a gift from Dr. Miguel Ondetti of the Squibb Institute for Medical Research, Princeton, NJ.

Animals. Male Sprague-Dawley rats were used throughout the experiments. The animals were kept at 23°C on a 12-h light-dark cycle and had free access to water and a standard laboratory diet. Rats were divided into three groups: (*a*) normal rats fed ad lib. (normal control) and normal rats fasted for 48 h with free access to water (fasted control); (*b*) chemically induced diabetic rats, both 24 h after streptozotocin (acute diabetic) and 15–19 d after streptozotocin or alloxan monohydrate injection (diabetic); and (*c*) insulin-treated diabetic rats.

Streptozotocin diabetes was induced by intravenous injection under ether anesthesia of 75 mg/kg streptozotocin into 24-h fasted rats as previously reported (16). Alloxan-diabetes was produced by similar intravenous injection of 50 mg/kg alloxan monohydrate into similarly fasted rats. To examine the chronic effect of diabetes on pancreatic exocrine function, the rats that gained weight at the rate of 0–20 g/wk were selected and used 15 to 19 d after injection. The average body weight gain after streptozotocin treatment was 2.08±0.14 g/d, whereas the normal rats gained 8.08±0.43 g/d. At the time of study, the diabetic rats weighed 110–170 g, whereas their normal littermates weighed 250–300 g. To investigate the effect of insulin treatment on diabetes, rats within the diabetic group were randomly chosen 14 d after streptozotocin administration and received Lente insulin daily for 7 d. 10 U of Lente insulin were given subcutaneously to the diabetic rats for the first 2 d. After that, the dosage of insulin was decreased to 8 U and then 2 d later to 6 U/d. During 7 d with insulin treatment body weight gain averaged 7.36±0.41 g/d.

Preparation of acini. Pancreatic acini from normal and diabetic rats of the same age fed ad lib. were prepared by the method of Williams et al. (15, 16). In brief, 1.1–1.3 g of pancreases were obtained from two rats between 9:30 and 10:30 a.m. to exclude any circadian variation. The isolated pancreases were injected by means of a 27-gauge needle with 5 ml Krebs-Henseleit bicarbonate buffer (KHB) containing 0.1 mM Ca^{2+}, 11.1 mM glucose, 0.1 mg/ml soybean trypsin inhibitor, 40–45 U/ml purified collagenase, 1.8 mg/ml hyaluronidase, and minimal Eagle's medium amino acid supplement.[2] To prepare acini from normal rats, 0.015 mg/ml chymotrypsin was added to the dissociation medium. Injected pancreatic tissue was incubated in a 25-ml polycarbonate Erlenmeyer flask at 37°C shaking 120 times/min. After 10 min, the enzyme solution was replaced with 5 ml fresh dissociation medium and the incubation continued for another 40 min. Acini were then mechanically dissociated by forceful pipetting through plastic pipettes with decreasing orifices and purified 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 Hepes-buffered Ringer's solution (HR). HR contained 10 mM HEPES, pH 7.4, as buffer, and was enriched with essential amino acids, 0.5% bovine serum albumin selected for low insulin-like activity, 0.01% soybean trypsin inhibitor, and was equilibrated with 100% O_2.

Enzyme content and secretion. In all experiments, acini were preincubated 60 min at 1.0–1.5 mg acinar protein/ml in HR at 37°C with shaking at 60 times/min. Acini were then centrifuged at 50 *g* for 2 min and resuspended in fresh HR at a density of 0.35–0.45 mg protein/ml. 2-ml aliquots were distributed into 25-ml polycarbonate Erlenmeyer flasks and secretagogues added.

Enzyme release in response to various doses of CCK_8 or carbamylcholine was determined using the procedure reported previously (15). At the beginning of each incubation,

[2] In some of the latter experiments hyaluronidase was omitted from the dissociation medium and replaced with bovine serum albumin. There was no difference in the functional properties of acini prepared with or without hyaluronidase.

TABLE I

Blood Glucose Serum Protein and Pancreatic Enzyme Contents in Normal and Chemically Induced Diabetic Rats

	Normal	Diabetes	
		Streptozotocin-induced	Alloxan-induced
Blood glucose, *mg/100 ml*	140±4 (32)	582±16 (73)	696±24 (15)
Serum protein, *g/100 ml*	8.4±0.2 (31)	7.7±0.1 (27)	8.0±0.3 (15)
Acinar amylase content, *U/mg protein*	79.25±4.41 (20)	0.31±0.04 (19)	0.57±0.1 (5)
Acinar ribonuclease content, *U/mg protein*	3.94±0.20 (23)	1.86±0.15 (24)	ND
Protein/DNA, *μg/μg*	33.1±1.4 (8)	26.6±1.2 (10)	34.1±1.5 (5)
Acinar amylase content, *U/mg DNA*	2,472.1±226.5 (8)	8.3±1.6 (10)	20.1±1.8 (5)
Acinar ribonuclease content, *U/mg DNA*	149.6±4.0 (8)	63.2±9.5 (10)	ND

Blood glucose values and serum protein concentrations are the mean±SE of the number of rats indicated in parenthesis. Amylase, ribonuclease, protein, and DNA concentrations in isolated acini are the mean±SE for the number of experiments indicated in parenthesis; in each experiment quadruplicate samples were assayed. ND, not determined.

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. 1 ml of water was added to each cell pellet and sonicated with a probe-type sonicator. Amylase and ribonuclease activities, and protein and DNA concentration were determined. Enzyme release was calculated relative to the protein concentrations of the initial acinar pellet and also as the percentage of the total content of the enzyme in the acinar pellet.

Intracellular transport and release of newly synthesized protein were analyzed using a radioassay according to Jamieson and Palade (17). After 30 min preincubation, acini were resuspended in fresh HR without leucine at an acinar density 4.5–5.5 mg protein/ml and pulse-labeled for 5 min with 20 μCi/ml 4,5-[^{3}H]leucine. At the end of the pulse, an excess of ice-cold HR containing 20 mM unlabeled leucine was added to the acinar suspension; labeled acini were centrifuged and washed again with the same ice-cold HR before resuspension at the usual acinar density (0.35–0.45 mg/ml) in freshly warmed HR (37°C) containing 0.4 mM leucine. Acini were then incubated for an additional 2 h in the presence or absence of CCK_8. Samples of incubation medium were removed at specified time points, and ribonuclease activity and the trichloroacetic acid precipitable radioactivity in the medium was determined. Release of newly synthesized ^{3}H-protein was expressed as percentage of the total ^{3}H-labeled protein initially present in the acini.

$^{45}Ca^{2+}$ efflux. Acini were preincubated for 45 min at 37°C in HR, then $^{45}CaCl_2$ (2 μCi/ml) was added and incubation continued for another 60 min. At the end of this loading period, the acini were centrifuged, washed once with ice-cold HR, and finally resuspended in fresh, nonradioactive medium that had been prewarmed to 37°C. 2-ml aliquots were distributed into 25-ml polycarbonate incubation flasks. Duplicate 0.5-ml aliquots were taken immediately (initial $^{45}Ca^{2+}$ content), different concentrations of CCK_8 or carbamylcholine added and further sampling of 1 ml from each flask was carried out after 5 min. The acini were separated from the extracellular medium by dilution into 10 vol of ice-cold saline with immediate suction through a 25-mm Nuclepore filter (Nuclepore Corp., Pleasanton, CA, 3 μm pore size) followed by a single wash with a further 5 vol of ice-cold saline. The filters containing acini were then placed in 12 × 75-mm plastic tubes, 2 ml water was added, and the tubes were sonicated with probe-type sonication. Radioactivity of the sonicated acini was determined by liquid scintillation counting. The $^{45}Ca^{2+}$ content of the acini was expressed as nanomoles per milligram protein. $^{45}Ca^{2+}$ efflux was expressed as the percentage of the radioactivity in the acini at the beginning of the incubation that was released over the 5-min incubation. The data was normalized by expressing the extra release of $^{45}Ca^{2+}$ over basal as a percentage of maximal release (set at 100%).

Assays. Amylase activity was determined by the method of Rinderknecht et al. (18) using amylose azure as substrate. Activity was expressed as porcine equivalents, using Sigma Chemical Co. amylase type 1-A as a standard. 1 U liberates 1 mg of maltose from starch in 3 min at pH 6.9 at 20°C. Ribonuclease activity was measured by the modified method of Anfinsen et al. (19) using yeast ribonucleic acid as substrate. Activity was expressed as bovine equivalents, using Sigma ribonuclease A from bovine pancreas (type X-A) as a standard.

Protein was assayed by the method of Lowry et al. (20) using bovine serum albumin as a standard. DNA was measured fluorometrically by the reaction between, 3,5-diaminobenzoic acid and deoxyribose sugar; calf thymus DNA was used as a standard (21). Serum glucose concentrations were measured by the hexokinase method (22) with the Worthington Statzyme glucose kit.

RESULTS

Enzyme activities in the diabetic pancreas (Table I). At the time of study the diabetic state in streptozotocin-treated rats was moderate to severe as manifested by the serum glucose concentration (582±16 mg/100 ml in streptozotocin-induced diabetes, vs. 140.1±3.7 mg/100 ml control). The amylase content in the acini prepared from streptozotocin-induced di-

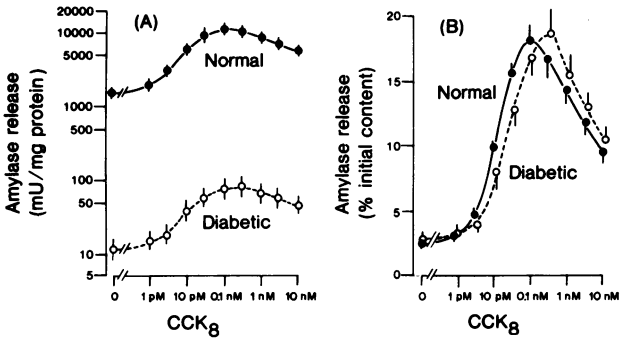

FIGURE 1 Concentration dependence of amylase release stimulated by CCK_8 from normal (●) and streptozotocin-induced diabetic (O) rat acini. Amylase release over 30 min is plotted as a function of the concentration of CCK_8 in the medium. (A) Amylase activity released into the medium expressed relative to the acinar protein concentration. (B) Amylase release expressed as the percentage of the total amylase activity initially present in the acini. Results shown are means±SE from four separate experiments in each of which amylase release was measured in triplicate incubation flasks at each concentration of CCK_8.

abetic rats was 0.31±0.04 U/mg protein, which was <1% of the content of acini from normal fed rats (79.25±4.41 U/mg protein). The acinar content of ribonuclease in diabetic rats was also decreased, but not to as great an extent as that of amylase. The ratio of total acinar protein to DNA in acini from streptozotocin-induced diabetic rats was significantly lower than that from normal rats (26.6±1.2 vs. 34.1±1.5; $P < 0.005$). Therefore, when pancreatic enzyme content was related to DNA, the large decreases were still seen in diabetic animals.

Response to secretagogues in streptozotocin diabetes. The initial release of digestive enzymes from acini provides a measure of secretagogue responsiveness that is independent of the preceding events in the synthesis and packaging of digestive enzymes, because it measures discharge of preformed exportable proteins stored within zymogen granules. When the amylase released during 30 min was studied as a function of the concentration of CCK_8, the same maximal ninefold increase was seen using the acini from both streptozotocin-treated diabetic and normal rats, even though basal amylase release was only 0.76% as great in diabetic acini (Fig. 1A). Fig. 1B is the normalized data for amylase release based on the initial content in the acini. Although acini from diabetic rats showed the same maximal responsiveness to CCK_8, expressed as percentage of initial content as acini from normal rats, they were less sensitive in terms of the threshold and maximally effective concentrations of CCK_8. A significant increase ($P < 0.05$) of amylase release from normal acini was observed with 1 pM CCK_8 and the maximal response was obtained with 0.1 nM CCK_8. On the other hand, the minimal and the maximal effective concentrations of CCK_8 for amylase release from diabetic acini were 3 pM and 0.3 nM, respectively. Both preparations showed reduced amylase release at supramaximal concentrations of CCK_8.

CCK_8 also increased the release of ribonuclease in acini from both normal and diabetic rats (Fig. 2A). The maximal percent release was comparable to that of amylase. As was the case for amylase release, a three times higher concentration of CCK_8 was required to induce the maximal ribonuclease release from the di-

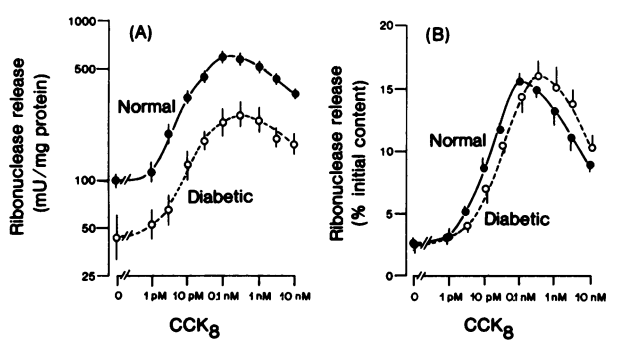

FIGURE 2 Concentration dependence of ribonuclease release stimulated by CCk_8 from normal (●) and streptozotocin-induced diabetic (O) rat acini. Ribonuclease release over 30 min is plotted as a function of the concentration of CCK_8 in the medium. (A) Ribonuclease release is expressed relative to the acinar protein concentration. (B) Ribonuclease release expressed as percentage of the total ribonuclease initially present in the acini. Results shown are means±SE from four separate experiments.

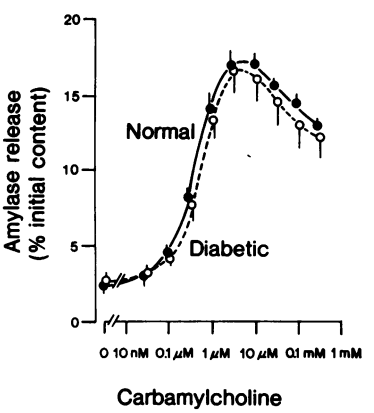

FIGURE 3 Concentration dependence of amylase release stimulated by carbamylcholine from normal (●) and streptozotocin-induced diabetic (O) rat acini. Amylase release over 30 min expressed as percentage of the total enzymatic activity initially present in the acini is plotted as a function of the added concentration of carbamylcholine. Results shown are means±SE from five separate experiments.

abetic acini as compared to normal acini, although the magnitude of the responses were the same (Fig. 2B).

Acetylcholine released from vagal nerve endings is the other major regulator of pancreatic acinar cell secretion. Cholinergic agonists act on a different receptor than CCK, but both appear to activate a common intracellular mechanism (23, 24). We therefore studied the ability of carbamylcholine to stimulate enzyme release in acini from diabetic and normal rats (Fig. 3). In contrast to CCK_8, the concentration dependence of carbamylcholine-stimulated amylase release from normal and diabetic acini was strikingly similar with the threshold concentration being 0.1 μM and the maximal response at 3 μM carbamylcholine.

Control studies. To determine whether these changes in diabetic acini were due to insulin deficiency or due to a direct effect of streptozotocin on exocrine cells, isolated acini were prepared from rats 24 h after streptozotocin injection. These rats showed elevated blood glucose levels (509.3±22.8, n = 8) and decreased acinar amylase content (55.7±1.4 U/mg protein). However, the responsiveness and the sensitivity to CCK_8 of these acini were the same as those of acini prepared from normal fed rats (Fig. 4). In addition, rats were injected with alloxan and after 14–19 d showed similar changes in blood sugar and acinar amylase content to rats injected with streptozotocin (Table I). Alloxan-injected rats did not appear to be as protein catabolic as rats injected with streptozotocin, however, since plasma protein and pancreatic protein/DNA ratios were normal. The dose-response curve to CCK_8 of acini prepared from alloxan-induced diabetic rats was shifted threefold to the right similar to that of streptozotocin-induced diabetic rats (Fig. 5). Moreover, the responsiveness to CCK_8 of these acini was decreased.

Because streptozotocin-induced diabetic rats selected for study gained body weight at the rate of 0–20 g/wk, a possibility was considered that the observed

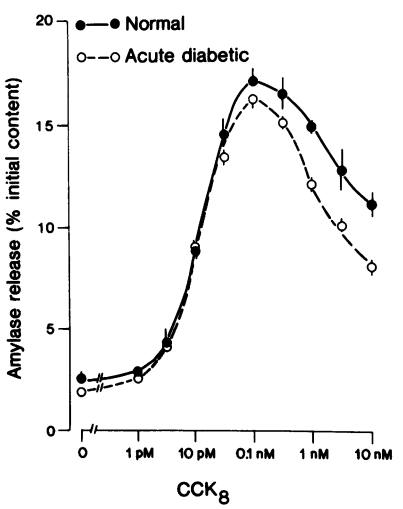

FIGURE 4 Concentration dependence of amylase release stimulated by CCK_8 from normal and acute diabetic rat acini. Amylase release over 30 min expressed as percent of the total enzymatic activity initially present in the acini is plotted as a function of the added concentration of CCK_8. Results shown are means±SE from four separate experiments.

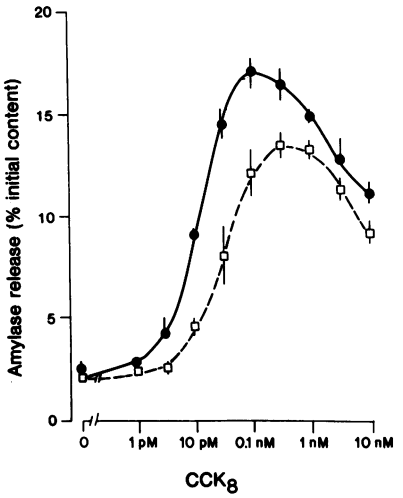

FIGURE 5 Concentration dependence of amylase release stimulated by CCK_8 from normal (●) and alloxan-induced diabetic (□) rat acini. Amylase release over 30 min expressed as percent of the total amylase activity initially present in the acini is plotted as a function of the added concentration of CCK_8. Results shown are means±SE from four separate experiments.

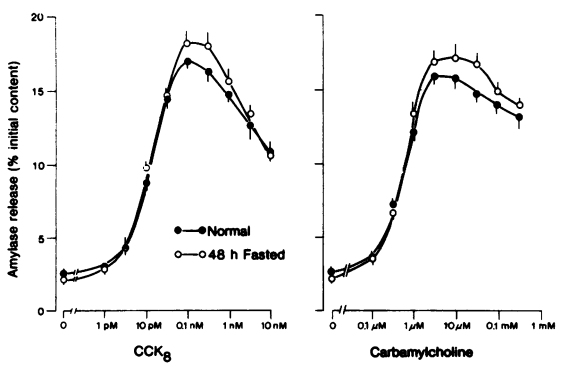

FIGURE 6 Concentration dependence of amylase release stimulated by CCK_8 and carbamylcholine from fed (●) and 48 h fasted (○) normal rat acini. Amylase release over 30 min expressed as percentage of the total amylase activity initially present in the acini is plotted as a function of the added concentration of CCK_8 (left panel) and carbamylcholine (right panel). Results shown are means±SE from four separate experiments.

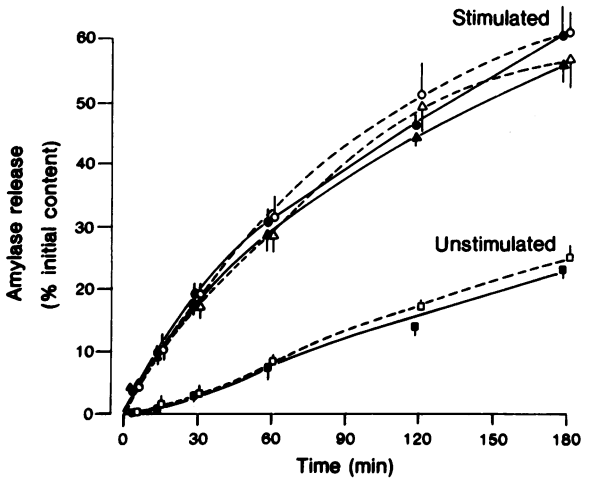

FIGURE 7 Time course of amylase secretion from normal and streptozotocin-induced diabetic rat acini. Control (■, □); 0.1 nM CCK_8 (●, ○); 3 μM carbamylcholine (▲, △). Solid symbols (■, ●, ▲) and solid lines represent the value from normal rat acini, while open symbols (□, △, ○) and dashed lines are from diabetic rat acini. Results shown are means±SE from four separate experiments.

changes in sensitivity to CCK_8 of acini from these diabetic rats were not due to insulin deficiency but due to malnutrition. To control for this possibility, rats were starved 48 h before study, over which period they lost 40.8±4.1 g (n = 8) of body weight. Amylase release from acini from these starved rats did not resemble the changes seen with diabetes. In the starved rats the sensitivity to CCK and carbamylcholine was unchanged, but the responsiveness was slightly increased (Fig. 6).

Time course of enzyme release. Pancreatic secretagogues normally bring about a sustained release of digestive enzymes. To determine whether the aforementioned results determined after a 30-min incubation were representative, we compared the time course of enzyme release by acini from diabetic and normal rats. CCK_8 (0.1 nM) and carbamylcholine (3 μM) stimulated amylase release from both normal and diabetic acini were maximal during the initial 5 min of incubation and then gradually decreased (Fig. 7). By 3 h, ~60% of the amylase initially present in the acini was released into the medium. Unstimulated amylase release was low up to 30 min but thereafter increased linearly with time. However no significant differences in the time course of control or secretagogue-stimulated amylase release from acini were observed between normal and diabetic rats. The time course of ribonuclease was similar to that of amylase release and was also similar when normal and diabetic acini were compared (data not shown).

Kinetics of intracellular transport. The appearance in the medium of proteins pulse-labeled with [^{3}H]leucine serves to determine the kinetics of intracellular transport, i.e., the time between protein synthesis and release of newly synthesized protein. Measurement of ribonuclease release was carried out in parallel on the same acinar preparation to determine the ability of these isolated acini to release preformed exportable proteins. Net discharge of ^{3}H-protein and ribonuclease above control during 2 h of incubation of acini prepared from diabetic and normal rats are shown in Fig. 8. Addition of CCK_8 (0.1 nM) to acinar suspensions immediately after pulse labeling induced the release of ribonuclease with discharge kinetics similar to those previously shown for amylase. After a lag of ~30 min, labeled proteins were released into the medium at a rate paralleling the release of ribonuclease. Both the minimal and average times for transport of labeled proteins in diabetic acini were the same as those in normal acini.

$^{45}Ca^{2+}$ efflux. The steps in stimulus-secretion coupling in response to CCK and cholinergic analogs can be divided into those leading to a mobilization of intracellular Ca^{2+} and those by which Ca^{2+} activates exocytosis. The effect on Ca^{2+} mobilization was evaluated by preloading acini with $^{45}Ca^{2+}$ and measuring the subsequent $^{45}Ca^{2+}$ efflux. CCK_8 increased $^{45}Ca^{2+}$ efflux from isolated acini from both normal and diabetic rats in a dose-dependent manner. One-half max-

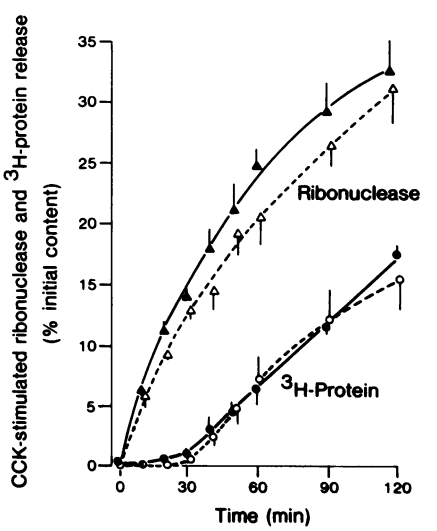

FIGURE 8 Discharge kinetics for newly synthesized proteins and ribonuclease from normal (●, ▲) and streptozotocin-induced diabetic (○, △) rat acini. Acini were pulse labeled with [^{3}H]leucine for 5 min and then incubated 120 min in response to 0.1 nM CCK_8. The values represent the net discharge of protein and ribonuclease activity. Control values have been subtracted from each stimulated value. Results shown are means±SE from four separate experiments.——, Normal; - - -, diabetic.

imal and maximal effective concentrations of CCK_8 for $^{45}Ca^{2+}$ efflux from normal acini were 50 pM and 1 nM, respectively, whereas those for $^{45}Ca^{2+}$ efflux from diabetic acini were 0.1 nM and 3 nM (Fig. 9). Thus, the dose-response curve in diabetic rats shifted two- to threefold to the right. On the contrary, the concentration dependence of carbamylcholine-stimulated $^{45}Ca^{2+}$ efflux from diabetic acini was shifted slightly to the left. Thus, the same differential loss of sensitivity was seen for CCK relative to carbamylcholine as for amylase release.

Effect of insulin treatment. After seven daily injections of insulin to streptozotocin-induced diabetic rats, the amylase content in the acini increased to >75% that of normal fed rats (60.6±7.1 in insulin-treated diabetic acini, 79.3±4.1 U/mg protein in control acini). The concentration-dependence of CCK_8-stimulated amylase release from insulin-treated diabetic rats was shifted threefold to the left compared with diabetic rats and was similar to that from normal fed rats with the maximal response at 0.1 nM (Fig. 10).

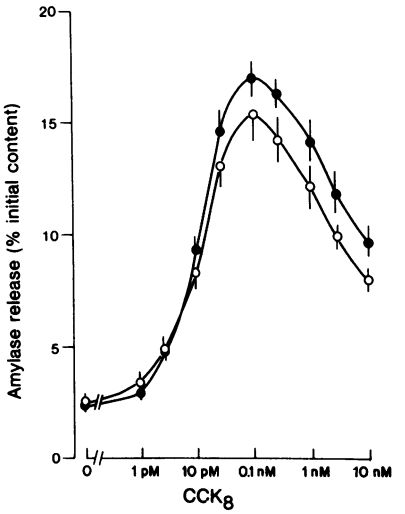

FIGURE 10 Concentration dependence of amylase release stimulated by CCK_8 from normal (●) and insulin-treated diabetic (○) rat acini. Amylase release over 30 min expressed as percentage of the total amylase activity initially present in the acini is plotted as a function of the added concentration of CCK_8. Results shown are means±SE from four to five separate experiments.

DISCUSSION

The three major phases in protein secretion by the exocrine pancreas are: (*a*) synthesis of digestive enzymes, (*b*) their intracellular transport, and (*c*) secretagogue-induced discharge of zymogen. The present study demonstrates that in diabetes the pancreatic content of digestive enzymes and the responsiveness to hormones are altered. The results of the present study, therefore, are in agreement with previous reports that indicated a decreased pancreatic content of amylase activity in diabetic rats (9–14). Moreover, the present investigation extends these observations by demonstrating that the pancreatic content of ribonuclease is also significantly reduced in diabetic acini. In this study, synthesis of digestive enzymes was not evaluated directly but previous studies have indicated that in rats the synthesis of amylase decreases in diabetes, whereas that of trypsinogen and chymotrypsinogen increases, and lipase remains unaltered (9–14). Because it is unlikely that a 100-fold fall in amylase content could be due to increased secretion or intracellular degradation in vivo, it seems most likely that this effect is also due to a decreased rate of synthesis. Recent data also indicate that the change in amylase content is paralleled by a change in specific pancreatic amylase messenger RNA content (25), suggesting that insulin regulates the synthesis of amylase at the level of transcription.

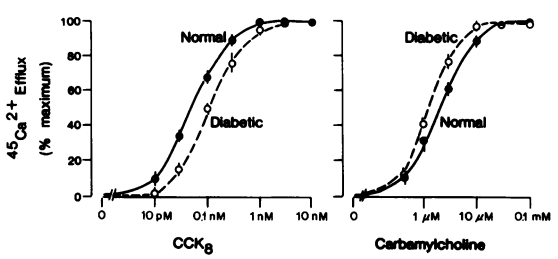

FIGURE 9 Concentration dependence of $^{45}Ca^{2+}$ efflux stimulated by CCK_8 and carbamylcholine from normal (●) and streptozotocin-induced diabetic (○) rat acini. $^{45}Ca^{2+}$ efflux over 5 min expressed as the percentage of the radioactivity in the acini at the beginning of the incubation is plotted as function of the added concentration of CCK_8 (left panel) and carbamylcholine (right panel). The data is normalized by expressing the extra release of $^{45}Ca^{2+}$ over basal as a percentage of maximal release. Results shown are means±SE from four separate experiments.

Intracellular transport appears normal in diabetic acini based on the discharge kinetics of newly synthesized protein after pulse labeling with [^{3}H]leucine. Specifically, the lag time from pulse labeling until newly synthesized protein is released into the medium was similar for normal and diabetic acini.

The maximal amounts of amylase and ribonuclease released in response to CCK and carbamylcholine were reduced in the acini from diabetic rats when results were expressed as a function of either acinar protein or DNA concentration. However, when results were expressed as the percentage of initial content of each enzyme in the acini, the secretory responsiveness of normal and diabetic acini for these enzymes was similar even though amylase and ribonuclease contents in acini from diabetic rats were <1 and 50%, respectively,

of normal. Thus the reduced maximal amounts of digestive enzymes released from acini prepared from diabetic rats is due to a reduced content of digestive enzymes rather than a reduced secretory capacity. However, acini from diabetic rats showed a decreased sensitivity to the secretagogue CCK, manifested by a threefold shift in the dose-response curve for both amylase and ribonuclease. A similar shift in the CCK dose-response curve has also been reported for [^{3}H]leucine incorporation into protein in acini from diabetic as compared with normal rats (26). The alteration in amylase release was a selective one since the sensitivity to the secretagogue carbamylcholine was not altered.

When the dose-response curves for $^{45}Ca^{2+}$ efflux (mobilization of cellular calcium) were studied, the predominant effect was also a loss of sensitivity to CCK. In contrast, the response to carbamylcholine was slightly potentiated. CCK and carbamylcholine are known to activate different receptors, and to subsequently activate a common pathway of calcium mobilization and action (23, 24). Our results with both amylase release and $^{45}Ca^{2+}$ efflux suggest that a primary effect of diabetes on pancreatic secretion is on the CCK receptor or receptor-activated transmembrane signaling before the convergence of later steps in CCK and carbamylcholine action.

Effects of streptozotocin on exocrine pancreas appear due to insulin deficiency, as they can be mimicked with alloxan-induced diabetes and reversed by exogenous insulin administration. It is possible, however, that some of the pancreatic abnormalities are due to alterations in nutritional or other hormonal states. Diabetic rats do not gain weight normally even though their food intake is increased (27). Although a significant positive correlation has been demonstrated between pancreatic enzyme output and the serum albumin concentration in protein-calorie malnutrition (28), serum protein concentrations in diabetic rats were only slightly decreased. Fasting rats for 48 h failed to mimic the effects of diabetes even though leading to considerable loss of body weight and a decrease in the nitrogen content of the pancreas (29). In any case, the lack of body weight gain in diabetes was secondary to insulin deficiency as it could be reversed by insulin administration.

Thus, the major functional abnormalities of pancreatic acinar cells from diabetic rats are an alteration of digestive enzyme content and a reduced sensitivity to CCK. These changes may be related to the observed abnormalities of exocrine pancreatic function in diabetic patients (1–5). Previous reports of pancreatic function in diabetic patients have demonstrated a low output of amylase during stimulation with CCK and secretin (1–5). Recently, an abnormally low output of trypsin and chymotrypsin in response to pancreatic stimulants has also been shown in juvenile diabetics (3, 4). It is not known whether this decreased pancreatic secretion is due to a decrease in the mass of normally functioning pancreatic acinar cells, a decrease in the content of specific digestive enzymes, a decrease in sensitivity to secretagogues or a combination of these factors. Many of the gastrointestinal manifestations of diabetes have previously been ascribed to diabetic neuropathy and autonomic insufficiency (30, 31). Although it should not be assumed that all the changes in diabetes are due to insulin lack, pancreatic acinar cells are now known to be regulated directly by insulin; acinar cells possess specific insulin receptors and insulin is reported to increase glucose transport and protein synthesis by acini, and to potentiate the secretagogue action of CCK (16, 26, 32–34). Thus, many of the exocrine pancreatic abnormalities in diabetes may be directly due to insulin deficiency. However, the role of structural changes in either the pancreatic vasculature or pancreatic parenchymal tissue (6–8, 35, 36), and changes in other hormones such as glucagon (37, 38), somatostatin (39, 40), and pancreatic polypeptide (41, 42) remain to be assessed. Furthermore, the reduced output of pancreatic bicarbonate in human diabetic (3, 4) suggests that pancreatic ductular functions as well as acinar function are abnormal. Unlike rats, most humans with diabetes do not have symptoms of severe pancreatic exocrine insufficiency such as steatorrhea (30, 31); more detailed pancreatic function tests should be carried out in man to determine if the sensitivity to individual hormones (CCK and secretin) is altered as was the case for CCK in the present study in the diabetic rat.

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