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Research Article

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Potentiation of Cholecystokinin-induced Exocrine Secretion by Both Exogenous and Endogenous Insulin in Isolated and Perfused Rat Pancreata

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ABSTRACT Using an isolated perfused rat pancreas preparation, the interrelationship between the endocrine and exocrine portions of the pancreas were studied. Addition of exogenous rat insulin (1–20 mU/ml) to the perfusing solution potentiated the action of cholecystokinin (CCK) (1 mU/ml) to increase both pancreatic juice flow and the release of the enzyme, amylase. Raising the glucose concentration in the perfusing solution from 2.5 to 17.5 mM both increased endogenous insulin release and potentiated the CCK-induced exocrine secretory response. Two lines of evidence indicated that this effect of glucose on the exocrine pancreas was mediated by endogenous insulin release. First, the addition of comparable amounts of xylose or galactose to the perfusion medium neither released insulin nor potentiated the CCK-induced response. Second, epinephrine blocked the effect of high glucose on both insulin release and potentiation of CCK action. Epinephrine alone did not affect the action of CCK. The magnitude of the exocrine response induced by high glucose was comparable to that of 2.5 mU/ml exogenous insulin. It seems possible that pancreatic acinar cells can be exposed to insulin levels of this magnitude *in situ*.

INTRODUCTION

In the pancreas of mammals, birds, and reptiles islets of endocrine tissue are scattered among the exocrine acini. Morphological studies reveal both cell-cell contact between these two types of tissue and direct connections between the capillaries of the islets and the acini (1–5). It has been hypothesized that these morphological arrangements reflect a regulatory role of the islet hormones in the function of the exocrine pancreas (1). Of the hormones, there is evidence that insulin controls pancreatic acinar cell function: In vivo insulin acts as a trophic factor to maintain the tissue level of amylase in acini of diabetic animals (6, 7) and in vivo also insulin influences the release of amylase by its hormonal stimulator, cholecystokinin (CCK)[1] (8, 9). Insulin in vitro stimulates glucose uptake by pancreatic acini and specific insulin receptors have been described (10). In the perfused rat pancreas insulin also potentiated the action of CCK (8, 11).

To date, however, all studies have been carried out with exogenous insulin. Whereas the exocrine pancreas was exposed to insulin in arterial blood, the possibility exists for a local effect of insulin at high concentration in an "islet-acinar portal system" (2). To test this possibility, we have stimulated the endogenous release of insulin by the in vitro perfused pancreas and studied its effect on CCK-induced exocrine pancreatic secretion. Comparison of these results with the response to exogenous insulin supports the concept that insulin has a physiological effect on acinar cells, and allows estimation of the *in situ* insulin concentration to which the acinar cells are exposed.

METHODS

Isolation and perfusion of the pancreas. All experiments were performed from June to September, 1978. Wistar strain male rats weighing about 250 g were fasted but allowed water for 24 h before the experiments. The isolated and perfused pancreas was prepared as reported (12). Briefly, under ether anesthesia, the vascular system was cannulated and perfused through the superior mesenteric and coeliac arteries, with the portal vein as outlet. The rate of vascular flow was kept constant at 4 ml/min by a roller pump in the experiments demonstrating the influence of intrinsic insulin, and 2 ml/min in the experiments demonstrating the influence of extrinsic insulin. The hepatic end of the common duct was ligated

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[1] *Abbreviation used in this paper*: CCK, cholecystokinin.

and the pancreatic juice was collected from the duodenal end of the duct after cannulation with a stainless steel tube. The blood supply to the stomach, liver, and spleen was stopped by tying the arteries. The mesentery, with its embedded whole pancreas and the attached duodenum, was then placed in a Lucite (E. I. Dupont de Nemours & Co., Inc., Wilmington, Del.) chamber containing 20 ml of a modified Krebs-Henseleit solution, maintained at 37°C.

Hormones and solution. The composition of the standard Krebs-Henseleit solution used for perfusing and bathing the preparation was as follows (mM): NaCl, 131; KCl, 5.6; $CaCl_2$, 2.5; $MgCl_2$, 1.0; $NaHCO_3$, 25; NaH_2PO_4, 1.0; and glucose, 2.5. Dextran T-70 (Pharmacia Fine Chemicals Inc., Uppsala) was added to the perfusing solution at a final concentration of 5% (wt/vol). Bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) was added at a concentration of 0.25%. The solution was equilibrated with 5% CO_2 in O_2 and had a pH of about 7.4. Natural CCK (99% pure, 3,500 U/mg, Gastro-Intestinal Hormone Research Unit, Karolinska Institute, Stockholm) was added to the solution perfusing the isolated pancreas. Its concentration is expressed in Ivy dog units (13) (1 mU/ml CCK = 286 pg/ml = 73 pM). In previous studies with the perfused rat pancreas, the threshold for CCK-induced pancreatic secretion was 0.2 mU/ml, the half-maximal response was about 1–2 mU/ml and maximal response at 5–10 mU/ml (14). Rat insulin (Novo Research Institute, Copenhagen, Denmark; 20.7 U/mg) was also added to the perfusing solution. *l*-Epinephrine bitartrate (Sigma Chemical Co.) was dissolved in 10 mM-HCl and added to the Krebs-Henseleit solution immediately before perfusion.

Estimation of digestive enzymes, flow of pancreatic juice, and insulin. The estimation of the flow rate of pancreatic juice was made as follows: A calibrated tube made of silicon-rubber (about 0.5 mm o.d.) was attached to the free end of the pancreatic duct cannula. At specified intervals the tube was replaced, and the rate of pancreatic juice flow down the tube measured, and the collected juice sample was diluted with a physiological saline solution at 2°C. The total protein in the pancreatic juice was assayed by the method of Lowry et al. (15), with bovine serum albumin as a standard. Amylase, in appropriately diluted samples, was assayed by the modified method of Bernfeld (16) as described (14). 1 U of amylase activity is defined as the amount of enzyme that produced 1 mg maltose during a 5-min incubation at 37°C. Insulin was assayed by a double antibody radioimmunoassay (Otsuka Assay Laboratory, Tokushima, Japan), which was calibrated against rat insulin standards dissolved in the perfusion buffer.

Statistics. Results are expressed as mean±SE, and are analyzed by Student's *t* test.

RESULTS

Potentiation of CCK action by exogenous insulin. Previous studies using the perfused rat pancreas have demonstrated that exogenous insulin, although having no effect by itself, would augment the CCK-induced secretion of pancreatic enzymes and juice (8) particularly by submaximal concentrations of CCK. By using an improved perfusion system containing bovine serum albumin as well as dextran, exogenous rat insulin was found to increase amylase released by 1 mU/ml CCK (Fig. 1). A detectable effect was seen at 1 mU/ml insulin and a maximal effect at 10 mU/ml insulin. Similar augmentation by insulin were seen for fluid and total protein secretion. Insulin alone had no effect on the basal secretion of amylase, fluid, or total protein.

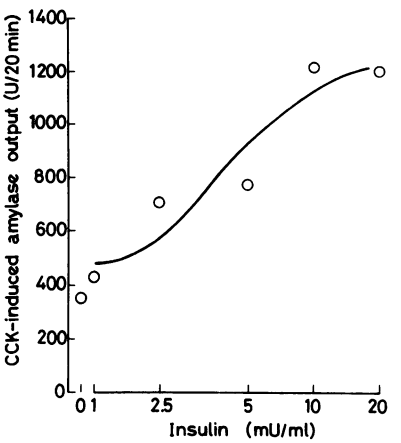

FIGURE 1 CCK-induced amylase output in response to the addition of rat insulin. Amylase release was summed over a 20-min period in response to 1 mU/ml CCK added 20 min after the addition of the specified amount of insulin. In the absence of CCK, amylase output was 23.41±2.43 U/20 min, and was not influenced by insulin. (26.15±2.60 U/20 min) Each value represents the mean of two to four pancreata.

Effects of glucose and other sugars on the CCK-induced secretory response. The isolated pancreas was first perfused for 20 min with the standard Krebs-Henseleit solution that contained 2.5 mM glucose to obtain the "resting" secretory responses of the exocrine and endocrine pancreas. The perfusion was then switched from the standard solution to a solution that contained 2.5 mM glucose with 1 mU/ml CCK and the perfusion continued for an additional 35 min (Fig. 2A). As expected CCK induced an increase in pancreatic juice flow as well as total protein and amylase output. There was no increase during the first 5-min exposure to CCK and a significant ($P < 0.05$) increase was observed for all samples after 10-min exposure. The total amount of CCK-induced protein output in the pancreatic juice collected during perfusion with the standard solution that contained 2.5 mM glucose was 810.4±151.6 μg/35 min. When the same concentration of CCK was added to medium that contained 17.5 mM glucose (Fig. 2B), the total protein output was 1,601.1 ±165.3 μg/35 min ($P < 0.01$ as compared to low glucose). The total amount of CCK-induced amylase output during perfusion with the standard solution was 918.9±211.6 U/35 min. In the presence of high glucose, the total amylase output was 1,875.2±217.1 U/35 min ($P < 0.01$). The addition of glucose alone had no consistent effect on any of the secretory parameters (Fig. 2). The total amount of CCK-induced juice flow during perfusion with the standard solution was 13.1±1.7 μg/35 min. In the presence of the high glucose, the total juice flow was 23.8±3.6 μl/35 min.

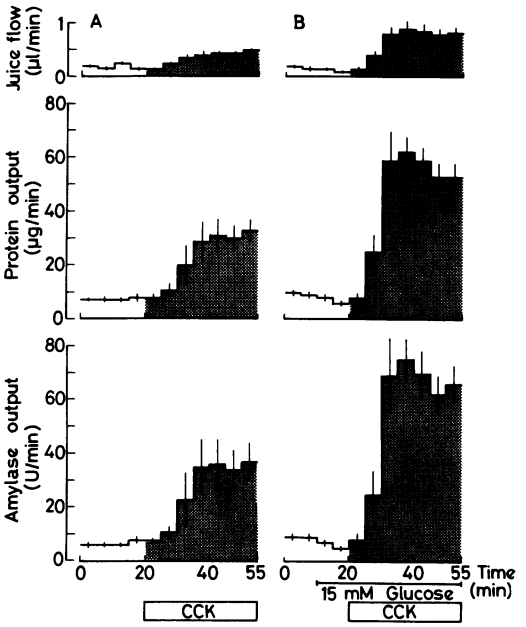

FIGURE 2 Effect of glucose on secretion of pancreatic juice, protein, and amylase by the perfused rat pancreas in response to CCK. (A) Pancreata were perfused with the standard solution containing 2.5 mM glucose. (B) Pancreata were perfused with the standard solution that contained 2.5 mM glucose from 0 to 10 min, at which point 15 mM additional glucose was added giving a total glucose of 17.5 mM. In all cases CCK (1 mU/ml) was added from 20 to 55 min. Each value represents the mean±SE of 5-min measurement from nine experiments.

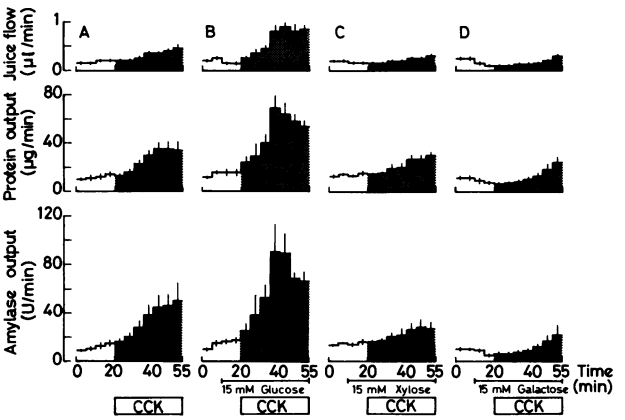

FIGURE 3 Effect of sugars on secretion of pancreatic juice, protein, and amylase in response to CCK. All pancreata were perfused with the standard solution that contained 2.5 mM glucose and 0.25% bovine serum albumin. From 10 to 55 min the following sugars were added at a concentration of 15 mM: (A) none, (B) glucose, (C) xylose, and (D) galactose. In all cases, CCK (1 mU/ml) was added from 20 to 55 min. Each value represents the mean±SE of a 5-min measurement from five experiments.

No potentiation of CCK-induced secretory responses was observed after the addition to the perfusing solution that contained 2.5 mM glucose of either 15 mM xylose or 15 mM galactose (Fig. 3). Moreover, the CCK-induced secretory response was slightly inhibited during perfusion with xylose or galactose.

Insulin release and the potentiating effect of glucose. Because it is well established that a high glucose concentration stimulates the pancreatic beta cells to release insulin, the potentiating effect of 17.5 mM glucose on CCK-induced exocrine secretion was most likely mediated by enhanced endogenous insulin release. In our preparation 17.5 mM glucose caused a biphasic insulin secretion, which was comparable to the results reported by Curry et al. (17). As shown in the Table I, this glucose-induced insulin release was not duplicated by the addition of xylose or galactose.

The comparison of glucose, xylose, and galactose suggests that an increase in sugar concentration in the perfusing solution may inhibit the CCK-induced secretory responses, but that this inhibitory effect of high sugar concentration may be overcome in the case of glucose by its stimulation of insulin release. To further test this hypothesis we studied the effect of epinephrine at 0.1 μM, a concentration that inhibits glucose-induced insulin release (Table I). Addition of epinephrine led to a sustained stimulation of fluid secretion and a transient release of total protein and amylase (Fig. 4). In the presence of 2.5 mM glucose and epinephrine, the subsequent addition of CCK was able to exert its usual secretagogue effects (Fig. 5A). When the same protocol was carried out in the presence of high glucose (but with insulin release inhibited by epinephrine) the effect of CCK was no longer potentiated but was actually inhibited (Fig. 5B).

DISCUSSION

In this study we have shown that both exogenous and endogenous insulin potentiates the action of secretagogues acting on the exocrine pancreas. Addition of bovine serum albumin to the perfusing solution has allowed demonstration of the action of exogenous rat insulin at a much lower concentration than reported in an earlier study (8, 9).

These studies emphasize the physiological relevance of insulin action on the exocrine pancreas by showing that endogenously released insulin has a similar effect. Because a single-pass perfusion system is used, insulin released from the beta cells must be acting on the exocrine cells prior to entering the pancreatoduodenal and portal veins. This is, to our knowledge, the first functional demonstration of the islet-acinus portal vessel system previously postulated on morphological grounds (1, 2, 18, 19). The comparison of the glucose potentiation of the response to CCK (approxi-

TABLE I

Insulin Released from the Perfused Pancreas during Studies of the Effect of Sugars and Epinephrine on CCK-induced Pancreatic Secretion

	Time		
	0–10 min	10–20 min	20–55 min
Solution that contained	2.5 mM Glucose	2.5 mM Glucose	2.5 mM Glucose + CCK
Perfusate insulin (3)	ND	ND	ND
Solution that contained	2.5 mM Glucose	17.5 mM Glucose	17.5 mM Glucose + CCK
Perfusate insulin (4)	ND	142±13 μU/ml	232±15 μU/ml
Solution that contained	2.5 mM Glucose + 0.1 μM epinephrine	17.5 mM Glucose + 0.1 μM epinephrine	17.5 mM Glucose + 0.1 μM epinephrine + CCK
Perfusate insulin (5)	ND	7±7 μU/ml	56±23 μU/ml
Solution that contained	2.5 mM Glucose	2.5 mM Glucose + 15 mM xylose	2.5 mM Glucose + 15 mM xylose + CCK
Perfusate insulin (4)	ND	ND	ND
Solution that contained	2.5 mM Glucose	2.5 mM Glucose + 15 mM galactose	2.5 mM Glucose + 15 mM galactose + CCK
Perfusate insulin (2)	ND	ND	ND

Insulin was measured in the portal vein effluent from the studies shown in Figs. 2 and 5. Basal samples were collected from 0 to 10 min, samples during perfusion with high sugar (17.5 mM) from 10 to 20 min, and samples during perfusion with high sugar plus CCK (1 mU/ml) from 20 to 55 min. Data obtained during the third collection period when CCK was present can not be compared to those during the second period when CCK is absent as the glucose-induced insulin release does not become maximal until 40–60 min. All values are the mean±SE of the number of experiments shown in parenthesis. The limit of detectability of insulin was 3 μU/ml. ND, nondetectible.

mately doubled; Figs. 1 and 2) to the potentiation in response to exogenous insulin suggests acinar cells after beta cell stimulation are exposed to an insulin concentration of 2-3 mU/ml. This is about 10 times higher than the insulin level in the portal vein during perfusion with 17.5 mM glucose (Table I). Because the

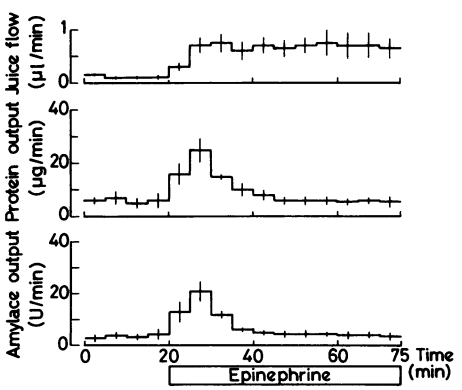

FIGURE 4 Transient increase in juice, protein, and amylase output from pancreata perfused with a solution containing epinephrine. Epinephrine (0.1 μM) was added after 10 min. Each value represents the mean±SE of a 5-min measurement from four experiments.

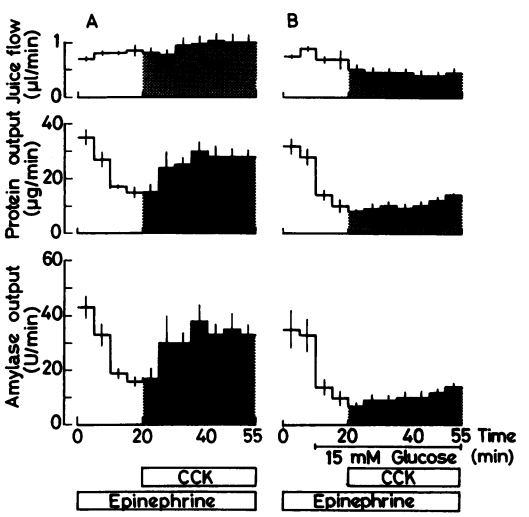

FIGURE 5 CCK-induced pancreatic juice, protein and amylase secretion from pancreata perfused with low and high concentration of glucose during exposure to epinephrine. In all cases epinephrine (0.10 μM) was added from 0 to 55 min and GCK (1 mU/ml) from 20 to 55 min. (A) Medium contains 2.5 mM glucose. (B) Medium contains 2.5 mM glucose from 0 to 10 min and thereafter 17.5 mM glucose. Each value represents the mean±SE of a 5-min measurement from five (A) or six (B) experiments.

pancreas and a portion of duodenum are being perfused, insulin levels in the portal vein thus would be expected to be lower than blood in the islet-acinar portal system. Whereas levels of insulin in the rat portal vein in vivo are not known, in vivo insulin levels in the superior pancreato-duodenal vein comparable to these have been reported in the dog (20, 21). In this case where the portal vein collects blood from a number of organs besides the pancreas, intrapancreatic insulin concentrations would be expected to be much higher than those in the portal vein.

Besides effects on insulin, changes in concentration of glucose in the perfusing solution may also effect release of glucagon or somatostatin although these were not measured in the present study. Somatostatin level has been reported to be significantly greater at higher glucose concentration in solution perfusing the isolated pancreas of the dog (22), and somatostatin has been reported to inhibit the response to CCK in the dog (23). Direct effects of glucagon on the exocrine pancreas, however, have not yet been established. Further work will be necessary to establish whether each of these islet hormones acts as a local regulator of the pancreatic acinar cells in the islet-acinar portal system (1, 2).

Previous work in the perfused rat pancreas has shown that exogenous insulin potentiates the ability of CCK to release amylase. In recent years it has become apparent that CCK also stimulates release of a Cl^--rich fluid, which is presumed to originate from acinar cells and is distinct from the juice secreted by the pancreatic ducts (24, 25). Because both exogenous and endogenous insulin potentiate pancreatic fluid as well as enzyme secretion, it is likely that insulin acts on a process common to both. Acetylcholine, acting on a distinct receptor, induces a pancreatic secretory response similar to CCK. Preliminary studies have shown that insulin also augments the secretory response to acetylcholine. The actions of both CCK and acetylcholine are believed mediated by intracellular Ca^{2+} as reviewed by Williams (26). Two possibilities for the mechanism of insulin are that it either increases intracellular Ca^{2+} mobilization, or the activity of a membrane Na^+–K^+ transport pump, which is important in secretion of both fluid and protein by the pancreatic acinar cells (14).

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