Increased β-Cell Secretory Responsiveness to Ceruletide and TPA in Streptozocin-Induced Mildly Diabetic Rats

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We examined the effects of various stimuli on immunoreactive insulin (IRI) and glucagon (IRG) release from perfused pancreases isolated from control and streptozocin-induced diabetic (STZ-D) rats. Diabetes was induced by injecting 30 mg/kg STZ into rats fasted for 16–18 h 12–17 days before our experiments. Glucose (11.1 mM) caused a distinct biphasic pattern of IRI release from the control pancreas, whereas the first phase was marginal and the second phase was absent in the diabetic pancreas. Arginine (20 mM)-induced IRI release was similar in both groups, whereas IRG release was greater in the control rats than in the diabetic rats. Thus, this model of STZ-D simulates a certain class of non-insulin-dependent diabetes mellitus (NIDDM). In these diabetic animals, the cholecystokinin (CCK) analogue ceruletide (620 pM) caused a significantly greater increase in IRI release in the presence of 5.6 mM glucose than in the control rats, but ceruletide caused a similar IRG release in both groups. Because CCK and ceruletide stimulate phosphoinositide turnover in pancreatic islets, we examined the effects of carbachol and phorbol ester TPA on IRI release in the presence of 5.6 mM glucose. Carbachol (10 μM), which is thought to generate similar second messengers as ceruletide, induced greater IRI release in diabetic than in control rats. TPA (100 nM) caused a significantly greater increase in IRI release from the diabetic than the control pancreas. Our results demonstrate that the insulin-releasing mechanism involved in protein kinase C activation is enhanced in this model of NIDDM. Considering the diminished glucose sensitivity of islets from STZ-D rats, the augmented insulinotropic effect of CCK may facilitate the maintenance of glucose homeostasis in these animals. *Diabetes* 38:1042–47, 1989

Glucagon	1 ng/L	= 1 pg/ml	Insulin	1 pM	= 0.139 μU/ml
Glucose	1 mM	= 18 mg/dl			

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The release of insulin from the pancreatic β-cell is regulated by metabolic substrates, hormones, and neurotransmitters. It has been shown that in certain groups of patients with non-insulin-dependent diabetes mellitus (NIDDM) and in various forms of experimental NIDDM, including spontaneous and streptozocin-induced diabetes (STZ-D), insulin release in response to glucose is impaired, whereas insulin release in response to arginine, leucine, acetylcholine, and isoproterenol is at least partially preserved (1–6). However, it is not precisely known how the insulinotropic action of various gastrointestinal hormones is influenced by diabetes. Several lines of evidence suggest that cholecystokinin (CCK) is a component of the enteroinsular axis. First, CCK and its related peptides stimulate insulin release in humans, dogs, and rats both in vivo and in vitro (7–12). Second, β-cells possess specific CCK receptors (13,14). Third, plasma CCK levels are elevated after oral administration of glucose in humans (15). Finally, a specific CCK-receptor antagonist attenuates meal-induced insulin release (16). Therefore, we investigated the effects of glucose, amino acid, and the CCK analogue ceruletide on insulin and glucagon release in control and STZ-D rats with the isolated perfused pancreas. Because CCK is suggested to stimulate insulin release through phosphoinositide turnover (17–20), we also examined the insulinotropic effects of carbachol and phorbol ester TPA. In this study, we used whole pancreases instead of isolated islets; it was technically difficult to prepare adequate numbers of isolated islets from diabetic rats.

RESEARCH DESIGN AND METHODS

Materials. STZ was purchased from Upjohn (Kalamazoo, MI); dextran 70 was purchased from Pharmacia (Uppsala, Sweden); bovine serum albumin (BSA; fraction V) was pur-

chased from Armour (Phoenix, AZ); arginine, carbachol (carbamylcholine chloride), and TPA (12-*O*-tetradecanoylphorbol-13-acetate) were purchased from Sigma (St. Louis, MO); rat insulin was purchased from Novo (Copenhagen); and glucagon 30K antiserum was purchased from the Univ. of Texas, Southwestern Medical School (Dallas). Synthetic ceruletide was kindly provided by Kyowa Hakko Kogyo (Tokyo).

Animals. Male Wistar rats were used in all experiments. The animals were kept at 23°C on a 12-h light-dark cycle. After a 16- to 18-h fast, the rats received 30 mg/kg STZ freshly dissolved in 0.01 M sodium citrate buffer (pH 4.5) via the tail vein under light ether anesthesia. Control rats received the citrate buffer. All rats were then fed ad libitum. After 12–17 days and a 16- to 18-h fast, the control and STZ-D rats were used for our experiments. This protocol resulted in mild diabetes in our previous studies in rats (21).

Immunoreactive insulin (IRI) and glucagon (IRG) in pancreas. Rats from both groups were killed by decapitation, and blood was collected for serum glucose concentrations. The entire pancreas was excised, weighed, and homogenized in acid ethanol with a glass Teflon homogenizer. Insulin and glucagon were then extracted from these homogenates by a modification of the method of Davoren (22).

In vitro isolated perfused pancreas. The isolated perfused pancreas was prepared as previously reported (11,23). Rats were anesthetized with ether after a 16- to 18-h fast, and the abdomen was opened. With ligation of appropriate blood vessels, the pancreas was separated from the colon, stomach, and spleen. The celiac and superior mesenteric arteries were inlets of vascular perfusion, and the portal vein was the outlet. The perfusate was Krebs-Ringer bicarbonate solution containing 46 g/L dextran 70, 2.5 g/L BSA, and 2.8, 5.6, or 11.1 mM glucose. It was equilibrated with 95% O_2/5% CO_2 and adjusted to pH 7.4. The rate of vascular perfusion was kept constant (2.0 ml/min) by a roller pump. Experiments were performed after at least 40 min of equilibration. For the evaluation of glucose-induced IRI release, 10 min of basal release was measured in the presence of 2.8 mM glucose, then the glucose concentration in the perfusate was increased to 11.1 mM by changing the medium reservoir. For the evaluation of other agonist-evoked IRI and IRG release, 5.6 mM glucose was used throughout the experiment, and 20 mM arginine, 620 pM (1 ng/ml) ceruletide, 10 μM carbachol, or 100 nM TPA was added for 10 or 20 min. We evaluated agonist-evoked IRI release in the presence of 5.6 mM glucose because 5.6 mM glucose did not significantly stimulate IRI release from the control rat pancreas. Each secretagogue was tested per pancreas, and these tests were repeated in 4–13 separate preparations of pancreas.

TABLE 1
Characteristics of control and streptozocin-induced diabetic rats

	Control	*n*	Diabetic	*n*
Body weight (g)				
Initial	230 ± 7	14	231 ± 4	12
Final	274 ± 7	14	248 ± 6*	12
Pancreatic weight (g)	1.01 ± 0.03	14	0.99 ± 0.04	12
IRI content (μg/pancreas)	53.5 ± 4.7	14	16.7 ± 3.4*	12
IRG content (μg/pancreas)	1.57 ± 0.26	7	1.94 ± 0.26	8
Fasting serum glucose concentration (mg/dl)	131 ± 4	14	189 ± 16*	12

Values are means ± SE of *n* rats. IRI, immunoreactive insulin; IRG, immunoreactive glucagon.
*$P < .05$ vs. control.

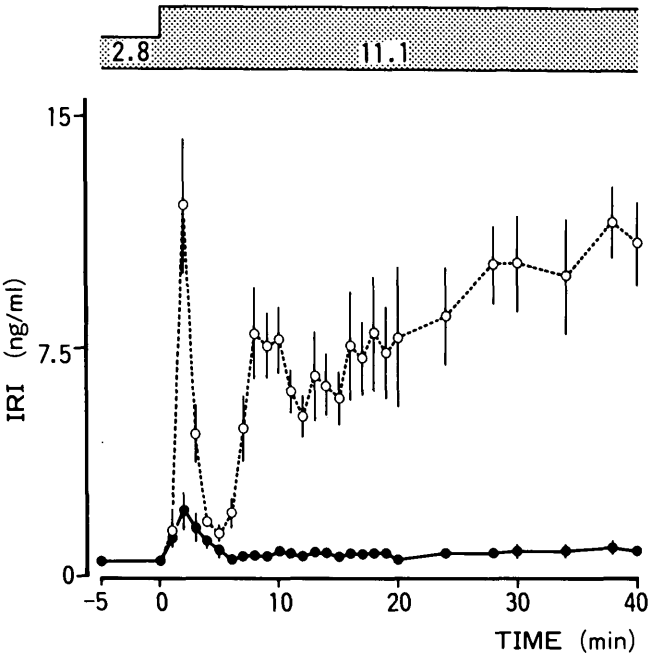

FIG. 1. Effect of 11.1 mM glucose (*shaded area*) for 20 min starting at time 0 on immunoreactive insulin (IRI) release from perfused pancreases isolated from control (○, *n* = 4) and diabetic (●, *n* = 5) rats fasted 16–18 h. One preparation of pancreas was used for each determination. Values are means ± SE.

Assays. IRI in the pancreatic extract and the portal effluent was measured by polyethylene glycol radioimmunoassay (24), and IRG was measured by charcoaled dextran radioimmunoassay with 30K antiserum (25). Rat insulin and porcine glucagon were used as reference standards in the IRI and IRG assays, respectively. Serum glucose concentrations were determined by the peroxidase method.

Data analysis. Results were expressed as means ± SE, and the differences in cumulative results were analyzed by Student's *t* test. *P* values $<.05$ were considered statistically significant.

RESULTS

Characteristics of animal groups. Pancreatic weight of the diabetic rats was not significantly different from that of the control rats, although the diabetic rats gained less weight than the control rats. Pancreatic IRI content of the diabetic rats was decreased by 70% compared with that of the control rats (Table 1). Although pancreatic IRG content of the diabetic rats was increased by 20%, this increase was not statistically significant. Fasting serum glucose concentrations of the diabetic rats were significantly higher than those of the control rats.

Effects of glucose on IRI release. After perfusion with 2.8 mM glucose, exposure of the control pancreas to 11.1 mM glucose significantly increased IRI release in a biphasic pattern (Fig. 1). In contrast, the diabetic pancreas responded to 11.1 mM glucose with only a small initial peak of IRI re-

lease, followed by levels of IRI release that were only barely measurable with our assay system (i.e., <0.5 ng/ml). Basal IRI release in the presence of 2.8 mM glucose was below the sensitivity of the assay in both groups.

Effects of arginine on IRI and IRG release. The addition of 20 mM arginine to perfusate containing 5.6 mM glucose caused a significant and biphasic IRI release from the control and diabetic pancreas (Fig. 2). Cumulative IRI output during 10 min of arginine stimulation was similar in both groups (Table 2). On the other hand, IRG release in response to arginine was greater in the control rats than in the diabetic rats.

Effects of ceruletide on IRI and IRG release. In the presence of 5.6 mM glucose, 620 pM (1 ng/ml) ceruletide caused a submaximal and significant increase in IRI release from the control pancreas (Table 3). As shown in Fig. 3, ceruletide stimulated IRI release in a biphasic pattern in control and diabetic pancreases. Ceruletide-stimulated IRI release was greater from the diabetic rat pancreas than from the control rat pancreas. Cumulative IRI output during 20 min of ceruletide stimulation was also significantly higher in diabetic than control rats (Table 2). On the other hand, IRG release in response to ceruletide was similar in two groups.

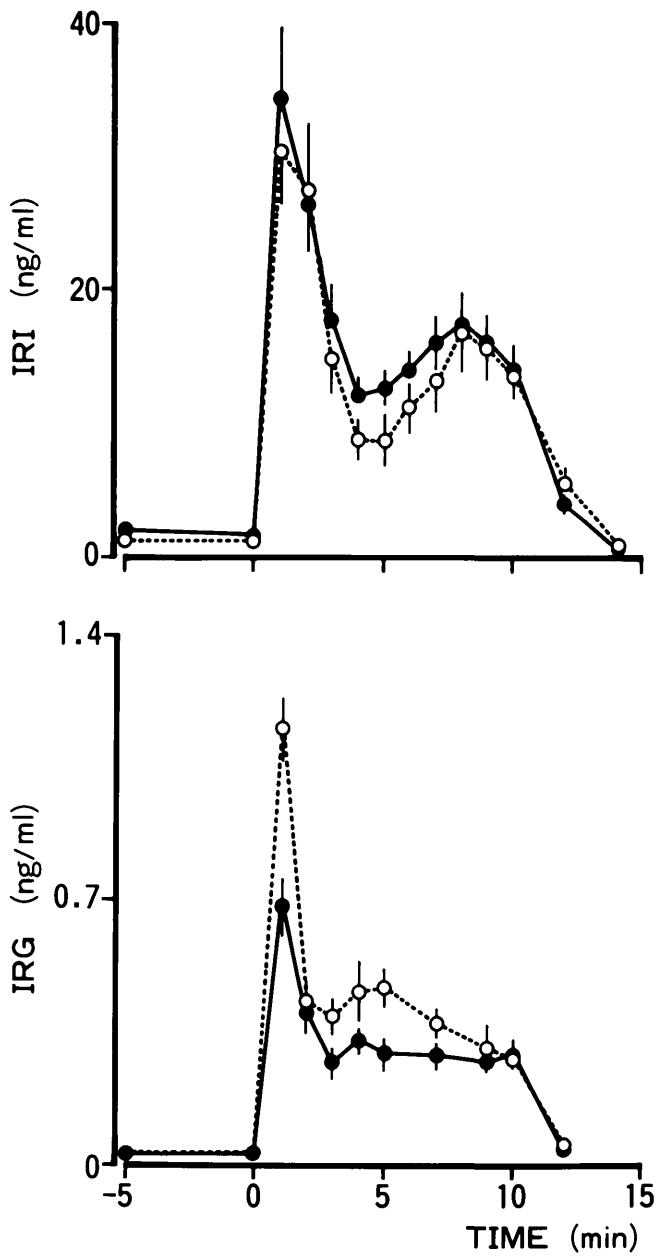

FIG. 2. Effect of 20 mM arginine for 10 min starting at time 0 on immunoreactive insulin (IRI) and glucagon (IRG) release in perfused pancreases isolated from control (○, *n* = 6) and diabetic (●, *n* = 8) rats fasted 16–18 h. Values are means ± SE.

TABLE 2
Effect of glucose, arginine, ceruletide, carbachol, and TPA on immunoreactive insulin (IRI) release from perfused pancreases isolated from control and diabetic rats

	Cumulative IRI output (ng/10 or 20 min)			
	Control	*n*	Diabetic	*n*
Glucose (11.1 mM)	240.2 ± 52.9	4	36.6 ± 5.7*	5
Arginine (20 mM)	322.1 ± 42.0	6	359.9 ± 47.1	8
Ceruletide (620 pM)	59.5 ± 11.4	13	143.6 ± 20.9*	8
Carbachol (10 μM)	257.0 ± 71.5	4	630.4 ± 84.5*	4
TPA (100 nM)	291.8 ± 48.5	4	791.2 ± 127.9*	4

Values are means ± SE of *n* experiments. Cumulative IRI output for glucose, ceruletide, carbachol, and TPA was derived for 20-min stimulation, and IRI output for arginine was derived for 10-min stimulation. When IRI value at any time dropped to an undetectable level, detection limit of the IRI assay (0.5 ng/ml) was used for calculation.
*$P < .05$ vs. control.

Effects of carbachol on IRI release. Because ceruletide and carbachol have common intracellular second-messenger systems (17–20,26), we examined the effects of carbachol on IRI release from the control and diabetic pancreas. In the presence of 5.6 mM glucose, 10 μM carbachol caused a significant biphasic IRI release from both groups. Like ceruletide, carbachol-stimulated IRI release was greater from diabetic rats than control rats (Fig. 4; Table 2).

Effects of TPA on IRI release. In pancreatic β-cells, protein kinase C is thought to play an important role in the stimulus-secretion coupling (27,28). Because CCK, ceruletide, and carbachol have been shown to cause phosphatidylinositol turnover in rat pancreatic islets (17–20), we examined the effects of TPA on IRI release from control and diabetic rat pancreases. Although TPA (100 nM)-stimulated IRI release was biphasic in both groups, release from diabetic pancreases was more rapid and lacked the time lag of control

TABLE 3
Effect of ceruletide on immunoreactive insulin (IRI) release from perfused pancreases isolated from control rats

Ceruletide (pM)	Cumulative IRI output (ng/20 min)
0	20.0
6.2	20.0
62	21.3 ± 0.8
310	38.5 ± 6.6*
620	59.5 ± 11.4*
3100	89.9 ± 19.0*
6200	107.8 ± 20.4*

Values are means ± SE of 4–13 experiments. Cumulative IRI output was calculated as indicated in Table 2.
*$P < .05$ vs. no addition of ceruletide.

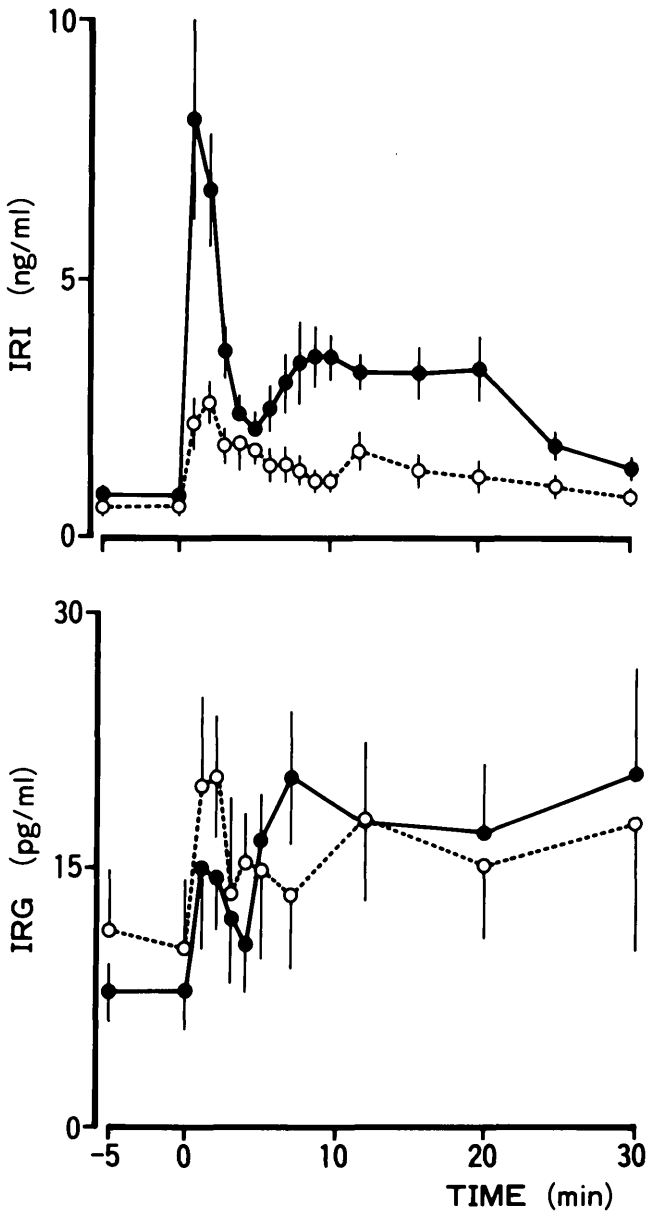

FIG. 3. Effect of 620 pM (1 ng/ml) ceruletide for 20 min starting at time 0 on immunoreactive insulin (IRI) and glucagon (IRG) release from perfused pancreases isolated from control (○, n = 13) and diabetic (●, n = 8) rats fasted 16–18 h. Values are means ± SE.

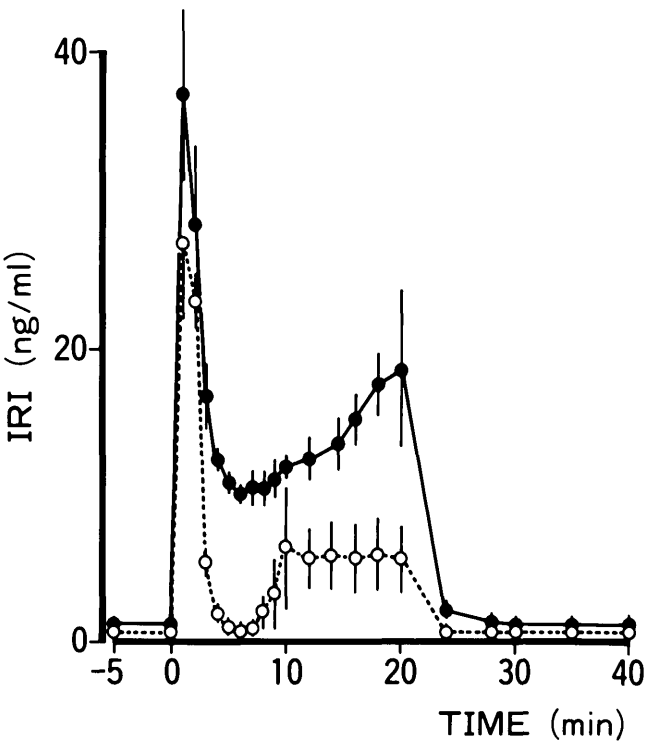

FIG. 4. Effect of 10 μM carbachol for 20 min starting at time 0 on immunoreactive insulin (IRI) release from perfused pancreases isolated from control (○, n = 4) and diabetic (●, n = 4) rats fasted 16–18 h. Values are means ± SE.

pancreases (Fig. 5). IRI output during 20 min of TPA stimulation was significantly higher in diabetic than control rats (Table 2).

DISCUSSION

This study demonstrates that ceruletide and TPA elicit significantly greater increases in insulin release from mildly diabetic rat pancreases than from control rat pancreases. CCK stimulates phosphatidylinositol turnover in rat pancreatic islets (17–20) and produces two intracellular mediators, diacylglycerol and inositol 1,4,5-trisphosphate. This results in Ca^{2+} mobilization and protein kinase C activation. TPA directly stimulates protein kinase C and elicits insulin release in the absence of glucose and enhances glucose-induced insulin release in pancreatic islets (27,29–31). In this study, 100 nM TPA caused an immediate increase and a subsequent progressive increase in insulin release from the isolated perfused pancreas. Both phases of insulin release were greater in diabetic than in control rats. A lower concentration of TPA (10 nM) significantly increased insulin release from the diabetic pancreas but elicited only marginal response from the control pancreas (data not shown). Although interpreting the results of the use of TPA to activate protein kinase C is difficult because of the lack of evidence that protein kinase C is the sole target for TPA (32), ceruletide-induced insulin release was also greater in diabetic than in control rats. Furthermore, the insulin-secretory response to carbachol, which also acts via phosphatidylinositol turnover, was increased in these diabetic rats. Therefore, the secretory responsiveness of β-cells to the protein kinase C inducers was increased in the mildly diabetic rats, which are a model of NIDDM.

Although the precise cellular mechanism by which insulin release through the protein kinase C pathway is increased in the diabetic rats is not known, there are possible explanations. Insulin release in response to combined TPA and calcium ionophore A 23187 is shown to be enhanced when cellular cAMP levels are elevated (27). It is speculated that cAMP may modulate the secretory response induced by secretagogues by potentiating the elevation in cytosolic Ca^{2+} and diacylglycerol (26). Recent studies have shown that cAMP phosphodiesterase activity is decreased in the tissue of STZ-D rats (33) and that the inhibitory activity of guanine nucleotide–binding regulatory proteins on adenylate cyclase is lost in the liver of STZ-D and alloxan-induced diabetic rats (34). These alterations can result in the elevation of cellular

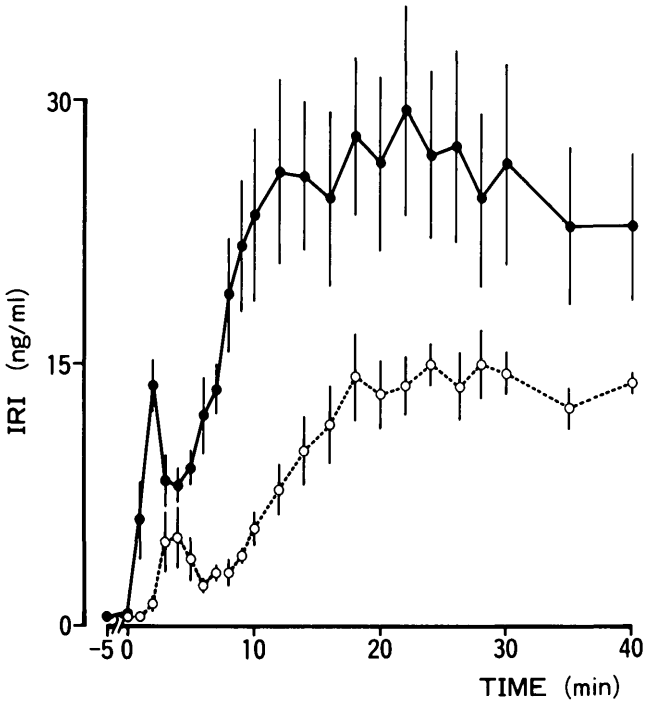

FIG. 5. Effect of 100 nM TPA for 20 min starting at time 0 on immunoreactive insulin (IRI) release from perfused pancreases isolated from control (○, *n* = 4) and diabetic (●, *n* = 4) rats fasted 16–18 h. Values are means ± SE.

cAMP levels. Similar alterations might have occured in pancreatic β-cells of the diabetic rats in our study and could have led to the enhanced insulin secretion in response to ceruletide, carbachol, and TPA in these animals. Glucagon also affects insulin release by increasing β-cell cAMP content. However, it is unlikely that glucagon caused the higher levels of insulin release from the diabetic pancreas; glucagon release in response to ceruletide was similar in both groups.

Another explanation for the enhanced insulin-releasing mechanism in the diabetic rats is that diabetes induces sensitization of pancreatic β-cells, because STZ-D rats are hyperglycemic, and hyperglycemia sensitizes islets to glucose. Previous glucose stimulation of pancreatic β-cells has been shown to amplify the insulin-secretory response to a subsequent agonist stimulation (35,36). This phenomenon was observed in nondiabetic animals and during a short duration after glucose exposure. In contrast, in rats with a reduced β-cell mass, it has been shown that prolonged hyperglycemia may lead to a defect in β-cell function by decreasing glucose-stimulated insulin secretion and impairing the modulating effect of nonglucose secretagogues on insulin secretion (4,5,37). However, it is possible that a sensitizing effect is involved in the mechanisms behind an enhanced β-cell response in the diabetic pancreas.

In the STZ-D rats, elevation of fasting serum glucose concentrations was mild, although pancreatic insulin content was decreased by 70%. Furthermore, glucose caused only a small increase in insulin release from the diabetic rat pancreas, whereas arginine elicited insulin release in the diabetic rats that was comparable with controls. Therefore, this model of experimental diabetes simulated NIDDM rather than insulin-dependent diabetes. In addition, the low-dosage STZ-D model in adult rats has the same characteristics as those manifested by NIDDM patients and the neonatal STZ-D model of NIDDM (i.e., decreased glucose-induced insulin response and insulin antagonism; 38,39). Although neonatal STZ-D was used as a model of NIDDM (4,5), this STZ-D model of NIDDM seems to possess potential as a useful, alternative model.

CCK is a possible incretin factor. The concept of incretin is derived from the fact that there is a difference between the insulin response after an oral load and the intravenous infusion of a similar amount of glucose. Although the precise identification of this incretin mechanism has not been established, several lines of evidence suggest a role for CCK in the enteroinsular axis (7–16). A recent report has shown that the intestinal insulinotropic effect was enhanced in diabetic rats (40). This study provides more evidence that the responsiveness of β-cells to ceruletide was increased in mildly diabetic STZ-D rats. Because this model of diabetes simulates NIDDM, our results suggest that CCK may play an important role as an incretin in at least a subclass of NIDDM. In NIDDM with compromised responsiveness of β-cells to glucose, insulin secretion may be compensated for by enhanced sensitivity of β-cells toward CCK and by possible polyphagia-induced increases in the release of CCK from the intestine.

REFERENCES

1. Deket T, Lauriden UB, Madsen SN, Deckert M: Serum insulin following isoprenalin in normal and diabetic persons. *Horm Metab Res* 4:229–32, 1972
2. Robertson RP, Porte D Jr: The glucose receptor: a defective mechanism in diabetes mellitus distinct from the beta adrenergic receptor. *J Clin Invest* 52:870–76, 1973
3. Palmer JP, Benson JW, Walter RM, Ensinck JW: Arginine-stimulated acute phase of insulin and glucagon secretion in diabetic subjects. *J Clin Invest* 59:837–48, 1976
4. Weir GC, Clore ET, Zmachinski CJ, Bonner-Weir S: Islet secretion in a new experimental model for non-insulin-dependent diabetes. *Diabetes* 30:590–95, 1981
5. Giroix MH, Portha B, Kergoat M, Bailbe D, Picon L: Glucose insensitivity and amino-acid hypersensitivity of insulin release in rats with non-insulin-dependent diabetes: a study with the perfused pancreas. *Diabetes* 32:445–51, 1983
6. Grill V, Herberg L: Glucose- and arginine-induced insulin and glucagon responses from the isolated perfused pancreas of the BB-Wistar diabetic rat: evidence for selective impairment of glucose regulation. *Acta Endocrinol* 102:561–66, 1983
7. Dupre J, Curtis JD, Unger RH, Wadell RW, Beck JC: Effect of secretin, pancreozymin or gastrin on the response of the endocrine pancreas to administration of glucose or arginine in man. *J Clin Invest* 48:745–57, 1969
8. Frame CM, Davidson MD, Sturdevant RAL: Effect of the octapeptide of cholecystokinin on insulin and glucagon secretion in the dog. *Endocrinology* 97:549–53, 1975
9. Otsuki M, Sakamoto C, Maeda M, Yuu H, Morita S, Baba S: Effect of caerulein on exocrine and endocrine pancreas in the rat. *Endocrinology* 105:1396–99, 1979
10. Sakamoto C, Otsuki M, Ohki A, Yuu H, Maeda M, Yamasaki T, Baba S: Glucose-dependent insulinotropic action of cholecystokinin and caerulein in the isolated perfused rat pancreas. *Endocrinology* 110:398–402, 1982
11. Okabayashi Y, Otsuki M, Ohki A, Sakamoto C, Baba S: Effects of C-terminal fragments of cholecystokinin on exocrine and endocrine secretion from isolated, perfused rat pancreas. *Endocrinology* 113:2210–15, 1983
12. Hermansen K: Effects of cholecystokinin (CCK)-4, non-sulfated CCK-8 and sulfated CCK-8 on pancreatic somatostatin, insulin, and glucagon secretion in the dog: studies in vitro. *Endocrinology* 114:1770–75, 1984
13. Sakamoto C, Goldfine ID, Roach E, Williams JA: Localization of saturable CCK binding sites in rat pancreatic islets by light and electron microscope autoradiography. *Diabetes* 34:390–94, 1985
14. Verspohl EJ, Ammon HPT, Williams JA, Goldfine ID: Evidence that cholecystokinin interacts with specific receptors and regulates insulin release in isolated rat islets of Langerhans. *Diabetes* 35:38–43, 1986

15. Liddle RA, Goldfine ID, Rosen MS, Tapelitz RA, Williams JA: Cholecystokinin bioactivity in human plasma: molecular forms, response to feeding and relationship to gallbladder contraction. *J Clin Invest* 75:1144–52, 1985
16. Rossetti L, Shulman GI, Zawalich WS: Physiological role of cholecystokinin in meal-induced insulin secretion in conscious rats: studies with L 364718, a specific inhibitor of CCK-receptor binding. *Diabetes* 36: 1212–15, 1987
17. Best L, Malaisse WJ: Phosphatidylinositol and phosphatidic acid metabolism in rat pancreatic islets in response to neurotransmitter and hormonal stimuli. *Biochim Biophys Acta* 750:157–63, 1983
18. Best L, Malaisse WJ: Nutrient and hormone-neurotransmitter stimuli induced hydrolysis of polyphospho-inositides in rat pancreatic islets. *Endocrinology* 115:1814–20, 1984
19. Zawalich WS, Takuwa N, Takuwa Y, Diaz VA, Rasmussen H: Interactions of cholecystokinin and glucose in rat pancreatic islets. *Diabetes* 36:426–33, 1987
20. Zawalich WS: Modulation of insulin secretion from β-cells by phosphoinositide-derived second-messenger molecules. *Diabetes* 37:137–41, 1988
21. Okabayashi Y, Ohki A, Sakamoto C, Otsuki M: Relationship between the severity of diabetes mellitus and pancreatic exocrine dysfunction in rats. *Diabetes Res Clin Pract* 1:21–30, 1985
22. Davoren PR: The isolation of insulin from a single cat pancreas. *Biochim Biophys Acta* 63:150–53, 1962
23. Okabayashi Y, Otsuki M, Ohki A, Nakamura T, Tani S, Baba S: Secretin-induced exocrine secretion in perfused pancreas isolated from diabetic rats. *Diabetes* 37:1173–80, 1988
24. Desbuquois B, Aurbach GD: Use of polyethylene glycol to separate free and antibody-bound peptide hormones in radioimmunoassay. *J Clin Endocrinol Metab* 33:732–38, 1971
25. Aguilar-Parada E, Eisentraut EM, Unger RH: Pancreatic glucagon secretion in normal and diabetic subjects. *Am J Med Sci* 257:415–19, 1967
26. Prentki M, Matschinsky FM: Ca^{2+}, cAMP, and phospholipid-derived messengers in coupling mechanisms of insulin secretion. *Physiol Rev* 67:1185–1248, 1987
27. Zawalich W, Brown C, Rasmussen H: Insulin secretion: combined effects of phorbol ester and A23187. *Biochem Biophys Res Commun* 117:448–55, 1983
28. Hubinont CJ, Best L, Sener A, Malaisse WJ: Activation of protein kinase C by a tumor promoting phorbol ester in pancreatic islets. *FEBS Lett* 170:247–53, 1984
29. Castagna M, Takai Y, Kaibuchi K, Sano K, Kikkawa U, Nishizuka Y: Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor-promoting phorbol esters. *J Biol Chem* 257:7847–51, 1982
30. Malaisse WJ, Sener A, Herchuelz A, Carpinelli AR, Polozcek P, Winand J, Castagna M: Insulinotropic effect of the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate in rat pancreatic islets. *Cancer Res* 40: 3827–31, 1980
31. Virji MAG, Steffes MW, Estensen RD: Phorbol myristate acetate: effect of a tumor promoter on insulin release from isolated rat islets of Langerhans. *Endocrinology* 102:706–11, 1978
32. Nishizuka Y: Studies and perspectives of protein kinase C. *Science* 233:305–12, 1986
33. Solomon SS, Deaton J, Shankar TP, Palazzolo M: Cyclic AMP phosphodiesterase in diabetes: effect of glyburide. *Diabetes* 35:1233–36, 1986
34. Gawler D, Milligan G, Spiegel AM, Unson CG, Houslay MD: Abolition of the expression of inhibitory guanine nucleotide regulatory protein Gi activity in diabetes. *Nature* (Lond) 327:229–32, 1987
35. Grill V: Time and dose dependencies for priming effect of glucose on insulin secretion. *Am J Physiol* 240:E24–31, 1981
36. Zawalich WS, Diaz VA, Zawalich KC: Role of phosphoinositide metabolism in induction of memory in isolated perifused rat islets. *Am J Physiol* 254:E609–16, 1988
37. Leahy JL, Bonner-Weir S, Weir GC: Abnormal glucose regulation of insulin secretion in models of reduced B-cell mass. *Diabetes* 33:667–73, 1984
38. Dall'Aglio E, Chang H, Hollenbeck CB, Mondon CE, Sims C, Reaven GH: In vivo and in vitro resistance to maximal insulin-stimulated glucose disposal in insulin deficiency. *Am J Physiol* 248:E312–16, 1985
39. Shen D-C, Davidson MB: Moderate insulinopenia can cause insulin antagonism. *Diabetes Res Clin Pract* 3:319–26, 1987
40. Ikeda T, Mokuda O, Kuno S, Tokumori Y, Tominaga M, Mashiba H: Enhanced intestinal insulinotropic effect in streptozotocin-diabetic rats. *Am J Physiol* 248:E304–308, 1985