Original Articles

Reduced Insulinotropic Effects of Glucagonlike Peptide I-(7–36)-Amide and Gastric Inhibitory Polypeptide in Isolated Perfused Diabetic Rat Pancreas

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The pathophysiological role of incretin in diabetes mellitus has not been established. We therefore examined the effects of glucagonlike peptide I-(7–36)-amide (truncated GLP-I) and gastric inhibitory polypeptide (GIP) on insulin and glucagon release from isolated perfused pancreases of diabetic rats (12–14 wk of age, mean ± SE fasting plasma glucose 8.9 ± 0.6 mM, *n* = 25) after an injection of 90 mg/kg streptozocin on the 2nd day after birth and compared the results with those of nondiabetic control rats. In diabetic rats, the infusion of 1 nM GLP-I or GIP in perfusates with varying glucose concentrations (2.8, 5.6, 8.3, 11.1, or 22.2 mM) caused a nearly equal degree of insulin stimulation from a similar basal insulin level. Meanwhile, basal and GLP-I– or GIP-stimulated insulin release increased in correlation with the ambient glucose concentration in nondiabetic rats. The degree of stimulation of insulin release at glucose concentrations of 5.6 mM in diabetic rats was ~33% that of nondiabetic rats. The stimulation potency was the same between GLP-I and GIP. The insulin treatment for diabetic rats (5 U/kg NPH insulin at 0900 and 2100 for 6 days) brought only a slight improvement in the glucose dependency of GLP-I–stimulated insulin release. The effects of GLP-I and GIP on glucagon release were completely opposite. GLP-I suppressed release; GIP stimulated it. In diabetic rats, the degree of suppression by GLP-I and stimulation by GIP were almost the same with similar basal glucagon levels in the perfusate with varying glucose concentrations. In nondiabetic rats, these parameters were inversely correlated with ambient glucose concentrations. Insulin treatment did not improve the glucose dependency of either the basal glucagon level or the degree of suppression by GLP-I. These data demonstrate that the incretin effect of GLP-I and GIP, glucose-dependent stimulation of insulin release, is reduced in neonatal streptozocin-induced diabetic rats and that conventional insulin treatment does not normalize this abnormality. Also, inhibition of glucagon release by GLP-I is glucose dependent in nondiabetic rats, and this glucose dependency is lost in diabetic rats. *Diabetes* 39:1320–25, 1990

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In response to food ingestion, many gastrointestinal hormones are released, and most of them modulate islet hormone secretion (1). On the physiological level, gastric inhibitory polypeptide (GIP) is a major stimulant of insulin release and has been called an incretin (2). Truncated glucagonlike peptide I-(7–36)-amide (GLP-I) has been demonstrated to possess a potent insulinotropic activity and has been proposed as an incretin candidate (3–5). The stimulatory effect of GIP on insulin release is dependent on the ambient glucose concentration for islet β-cells, which is one of the prerequisites to be an incretin (6,7). However, the glucose dependency of insulin release by GLP-I has not been fully documented (8,9).

In addition, the response of β-cells to glucose is extremely reduced in non-insulin-dependent diabetes mellitus (NIDDM; 10,11). Consequently, it is presumed that the incretin effect is also decreased in NIDDM. Krarup et al. (12) demonstrated that insulin release in response to GIP infusion was impaired in diabetic patients. However, no precise study regarding the incretin effect in diabetes mellitus has been carried out.

We therefore examined the glucose dependency of the stimulation of insulin release brought on by GLP-I and GIP in nondiabetic rats and rats with streptozocin-induced diabetes (STZ-D) in the neonatal period. The glucose dependency of the modulation of glucagon release was also examined, because the glucose dependency of glucagon release by GLP-I has not been reported.

RESEARCH DESIGN AND METHODS

GLP-I was synthesized by the stepwise solid-phase method with a Beckman 990-B automatic synthesizer (Fullerton, CA) and then purified by high-performance liquid chromatography (HPLC). The purity of the peptides was monitored by analytical reverse-phase HPLC on a column of Nucleosil 5C-18 (4.6 × 150 mm; Gaskurokogyo, Tokyo) under the isocratic conditions of 0.1% trifluoroacetic acid and 39% acetonitrile and proved to be at least 98% pure (13). Human GIP was purchased from Peptide Institute (Osaka, Japan).

Two-day-old Wistar rats were injected with 90 mg/kg i.p. STZ (Sigma, St. Louis, MO). Rats receiving the diluent (0.05 M sodium citrate, pH 4.5) were used as nondiabetic controls. At 10 wk of age, an oral glucose tolerance test (2 g/kg body wt) was performed. Blood was obtained by tail snipping before and 60 and 120 min after glucose loading. The mean ± SE plasma glucose concentrations of diabetic rats in this study were as follows: before, 8.9 ± 0.6 mM; 60 min, 23.0 ± 1.2 mM; 120 min, 15.6 ± 1.4 mM (n = 25). Their body weights were less than those of nondiabetic controls (332 ± 10 vs. 430 ± 10 g, $P < 0.01$).

At 13 wk of age, some of the diabetic rats were treated with 5 U/kg s.c. intermediate-acting insulin (Humulin N, Shionogi-Lilly, Osaka, Japan) at 0900 and 2100 for 6 days. The last injection was administered at 0900 1 day before the pancreas perfusion was performed.

The pancreases were isolated from male STZ-D and control rats at 12–14 wk of age under pentobarbital anesthesia after an overnight fast. The isolated rat pancreases were perfused by the method of Grodsky et al. (14). A Krebs-Ringer bicarbonate buffer solution containing 4% dextran T-70 (Pharmacia, Uppsala, Sweden); 0.2% bovine serum albumin; and 5 mM each of pyruvate, fumarate, and glutamate was equilibrated with a 95% O_2/5% CO_2 mixture at 37°C and then continuously gassed throughout the experiment. After 25 min of equilibration, sampling was performed for 30 min (Figs. 1–4), and then the second 30 min of sampling was performed after 15 min of equilibration, including changing of the glucose concentration of the perfusate by means of a sidearm syringe for the second experiment. Therefore, the total perfusion period was 100 min. The flow rate of the perfusate was 2 ml/min, and the peptide solution was administered through a sidearm syringe at a rate of 0.1 ml/min. Because prior exposure of the pancreas to one agent could influence the subsequent response to another challenge, the order in which the agents were given was randomized in separate experiments. The effluent perfusate was collected at 1-min intervals and stored at −40°C until the assay.

Plasma glucose concentration was measured with a Beckman Glucose Analyzer II. Immunoreactive insulin (IRI) and immunoreactive glucagon (IRG) in the effluent perfusate were determined by radioimmunoassay according to the methods of Herbert et al. (15) and Faloona and Unger (16), respectively, with E-7 antibody (17; kindly donated by H. von Schenck).

Analysis of variance and the Newman-Keul's procedure

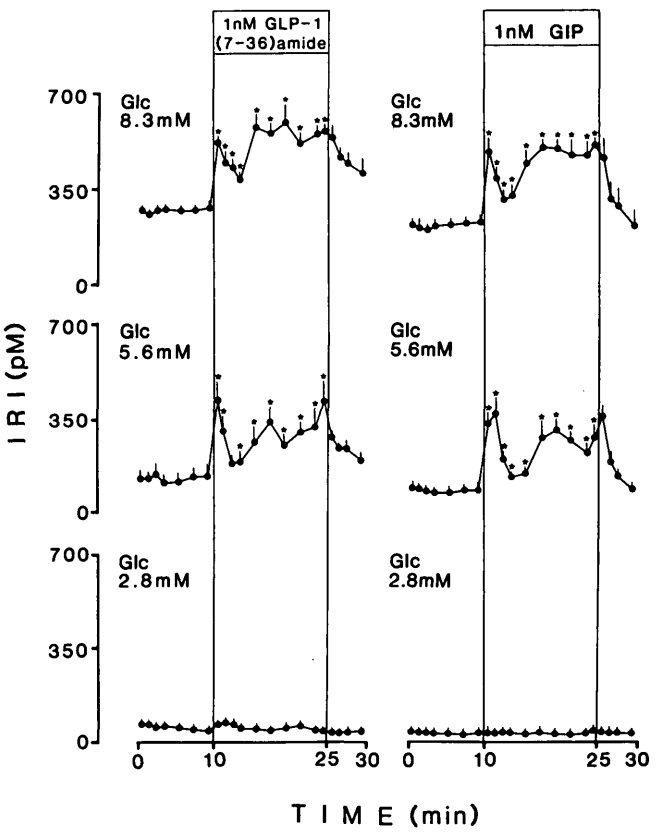

FIG. 1. Effects of perfusate glucose (Glc) concentration on stimulation of immunoreactive insulin (IRI) release by 1 nM glucagonlike peptide (GLP) I-(7–36)-amide and gastric inhibitory polypeptide (GIP) from isolated perfused nondiabetic rat pancreas. Values are means ± SE (n = 4–5). *$P < 0.05$ vs. preceding baseline.

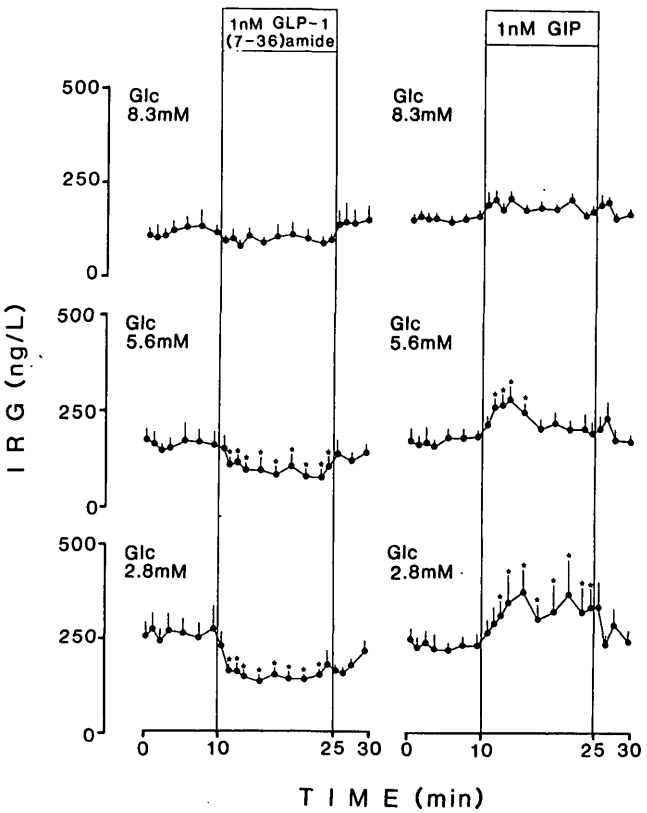

FIG. 2. Effects of perfusate glucose (Glc) concentration on modulation of immunoreactive glucagon (IRG) release by 1 nM glucagonlike peptide (GLP) I-(7–36)-amide and gastric inhibitory polypeptide (GIP) from isolated perfused normal rat pancreas. Values are means ± SE (n = 4–5). *$P < 0.05$ vs. preceding baseline.

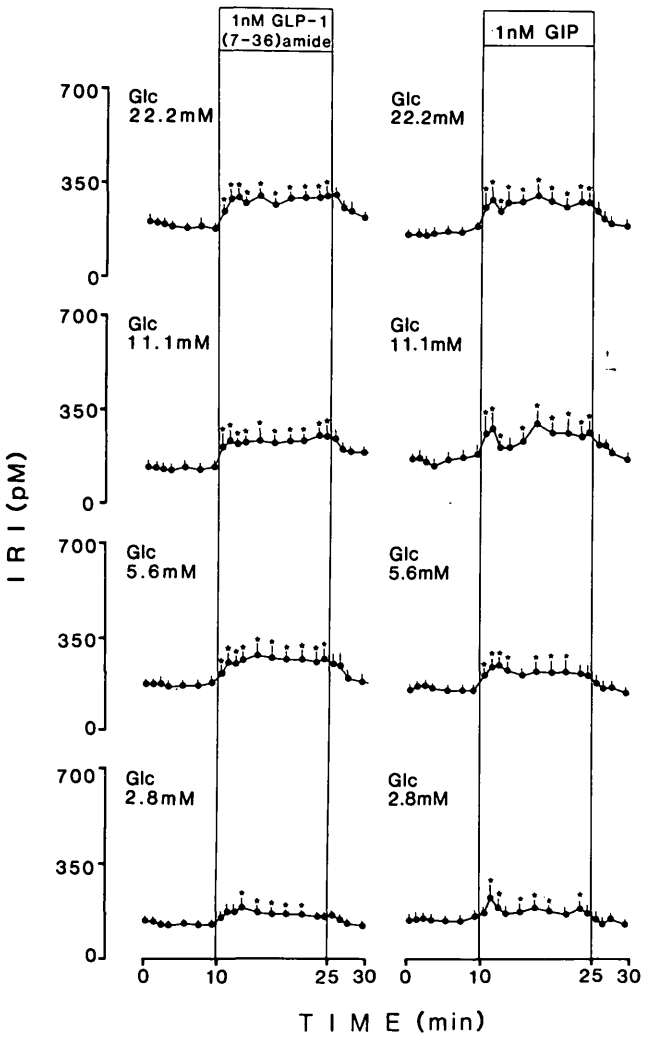

FIG. 3. Effects of perfusate glucose (Glc) concentration on stimulation of immunoreactive insulin (IRI) release by 1 nM glucagonlike peptide (GLP) I-(7–36)-amide and gastric inhibitory polypeptide (GIP) from isolated perfused diabetic rat pancreas. Values are means ± SE (n = 6–7). *$P < 0.05$ vs. preceding baseline.

for multiple comparisons were performed (Figs. 1–4). The net changes in insulin and glucagon release during the infusion of peptide were compared with the basal levels by Student's t test for paired data after measuring the area under the curves of insulin or glucagon level in the perfusate (Figs. 5 and 6). The net increase in insulin release between the insulin-treated group and the untreated group was compared by Student's t test for unpaired data (Fig. 7). $P < 0.05$ was considered significant, and all data are expressed as means ± SE.

RESULTS

Nondiabetic rats. The basal IRI level increased in parallel with the perfusate glucose concentration (38.1 ± 2.9 pM at 2.8 mM, 79.7 ± 9.3 pM at 5.6 mM, and 211.1 ± 13.6 pM at 8.3 mM in the GIP experiment; Fig. 1). Neither 1 nM GLP-I nor GIP significantly stimulated insulin release under the perfusate glucose concentration of 2.8 mM (Fig. 1). During perfusion with 5.6 and 8.3 mM glucose, significant stimulation of insulin release was observed in a biphasic pattern by both 1 nM GLP-I and GIP. The degree of stimulation was the same with 1 nM GLP-I and GIP. The degree of net increase from the basal level with both hormones was clearly dependent on the glucose concentration of the perfusate (Fig. 5).

The basal IRG level decreased in parallel with the perfusate glucose concentration (258 ± 46 ng/L at 2.8 mM, 157 ± 27 ng/L at 5.6 mM, and 108 ± 28 ng/L at 8.3 mM in the GIP experiment; Fig. 2). GLP-I (1 nM) caused significant suppression of glucagon release at 2.8 mM perfusate glucose. When the glucose concentration was 5.6 mM, the degree of suppression decreased, and no significant changes were observed at 8.3 mM (Figs. 2 and 6).

On the other hand, 1 nM GIP clearly stimulated glucagon release at 2.8 mM glucose. The degree of stimulation gradually decreased with a negative correlation to the ambient glucose concentration (Figs. 2 and 6).

Diabetic rats. The basal IRI level did not increase in parallel with the increase in perfusate glucose concentration (178.1 ± 26.6 pM at 11.1 mM and 168.7 ± 17.2 pM at 22.2 mM in the GIP experiment; Figs. 3 and 7). A slight but significant increase in IRI level was observed with 1 nM GLP-I

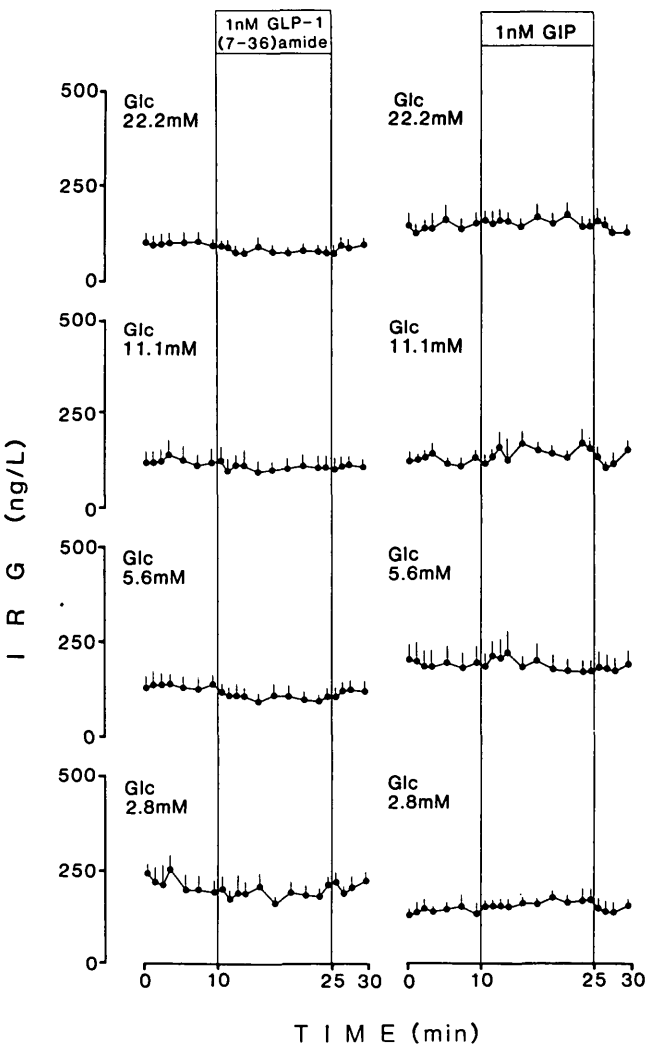

FIG. 4. Effects of perfusate glucose (Glc) concentration on modulation of immunoreactive glucagon (IRG) release by 1 nM glucagonlike peptide (GLP) I-(7–36)-amide and gastric inhibitory polypeptide (GIP) from isolated perfused diabetic rat pancreas. Values are means ± SE (n = 6–7).

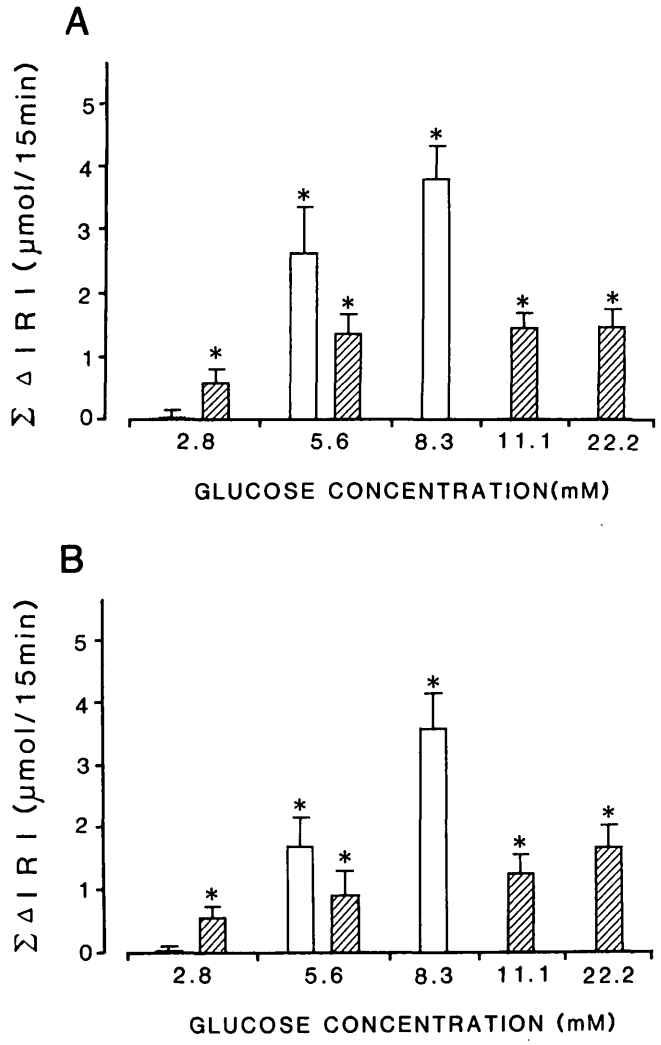

FIG. 5. Net increase in insulin release during infusion of 1 nM glucagonlike peptide-I-(7–36)-amide (*A*) and 1 nM gastric inhibitory polypeptide (GIP; *B*). Σ Δ immunoreactive insulin (ΣΔIRI; μmol/15 min) was calculated as net incremental area of insulin release during infusion of peptides from basal level. *Open bars*, nondiabetic control rats; *hatched bars*, diabetic rats. Values are means ± SE. Number of experiments is shown in Figs. 1 and 3. *$P < 0.05$ vs. preceding baseline.

at 2.8 mM glucose. The degree of stimulation increased at 5.6 mM glucose, and a similar degree of stimulation was obtained at 11.1 and 22.2 mM glucose (Figs. 3 and 5).

GIP (1 nM) also significantly stimulated insulin release at 2.8 mM glucose. In parallel with the increase in glucose concentration, the degree of stimulation gradually increased, but the changes were very small, and no significant difference in increase was observed between 5.6 and 22.2 mM perfusate glucose (Fig. 5).

At 5.6 mM glucose, the degree of net increase in insulin release by GLP-I or GIP in diabetic rats was ~50% that in nondiabetic rats (Fig. 5).

The basal IRG level did not decrease in parallel with the perfusate glucose concentration (136 ± 17 ng/L at 2.8 mM and 191 ± 41 ng/L at 5.6 mM in the GIP experiment; Fig. 4). No significant suppression of glucagon release was observed by infusion of 1 nM GLP-I at any glucose concentration of the perfusate tested, although it tended to decrease glucagon release (Figs. 4 and 6). The infusion of 1 nM GIP also did not cause any significant increase in the IRG level of the perfusate at 2.8, 5.6, 11.1, and 22.2 mM glucose, although it tended to increase glucagon release (Figs. 4 and 6).

Table 1 shows the plasma glucose concentrations of diabetic rats on the 4th day of insulin treatment. Before the insulin injection (0900), the plasma glucose concentration was 13.3 ± 1.4 mM (n = 23). Two hours after the injection of insulin, it decreased to 5.4 ± 0.5 mM. Thereafter, the plasma glucose level gradually increased and returned to its initial level 10 h after the injection.

Figure 7 shows the basal IRI level and the net changes of insulin release by 1 nM GLP-I from the isolated perfused pancreas of untreated diabetic rats and diabetic rats treated with insulin. The basal IRI level seems to recover its dependency on the perfusate glucose concentration (*hatched bars*), but it is not statistically significant by linear regression analysis. The net increase in insulin release recovered its

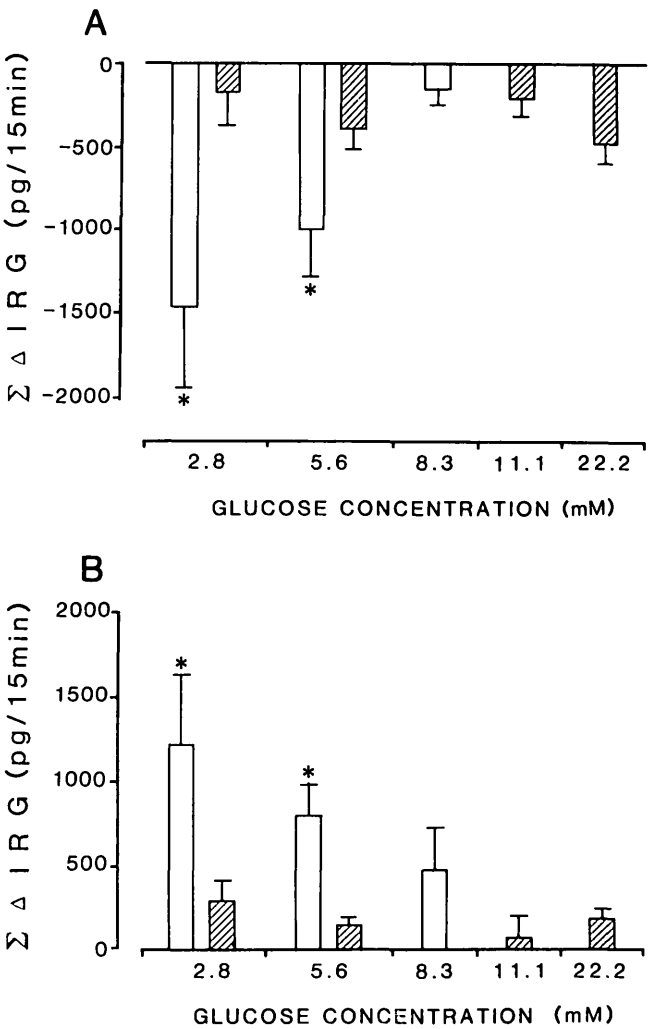

FIG. 6. Net decrease in glucagon release during infusion of 1 nM glucagonlike peptide-I-(7–36)-amide (*A*) and its net increase during infusion of 1 nM gastric inhibitory polypeptide (*B*). Σ Δ immunoreactive glucagon (ΣΔIRG; pg/15 min) was calculated as area of glucagon release during infusion of peptides from basal level. *Open bars*, nondiabetic control rats; *hatched bars*, diabetic rats. Values are means ± SE. Number of experiments is shown in Figs. 2 and 4. *$P < 0.05$ vs. preceding baseline.

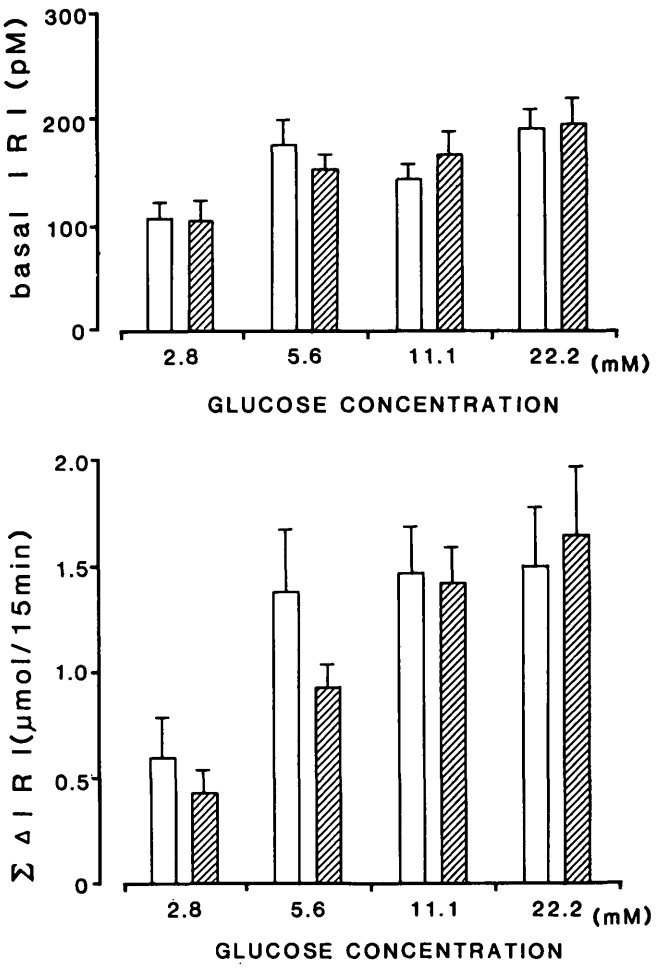

FIG. 7. Effects of insulin treatment on basal immunoreactive insulin (IRI) level and stimulation of IRI release (ΣΔIRI) by 1 nM glucagonlike peptide-I-(7–36)-amide from isolated perfused diabetic rat pancreas at varying ambient glucose concentrations. *Open bars*, results from untreated diabetic rats; *hatched bars*, results from insulin-treated diabetic rats. Values are means ± SE (*n* = 5–7).

dependency on the perfusate glucose concentration ($P < 0.05$ by linear regression analysis). However, no significant difference in net increase in insulin release was found between the insulin-treated group and the untreated group at any glucose concentration of perfusate. Concerning glucagon release, neither basal IRG level nor the response to GLP-I was normalized by insulin treatment (data not shown).

DISCUSSION

In this study, we demonstrated that *1*) the modulation of insulin and glucagon release from the isolated perfused nondiabetic rat pancreas by GLP-I or GIP is clearly dependent on the glucose concentration of the perfusate; *2*) GLP-I is compatible with the definition of incretin at this point (18), and GIP and GLP-I have the same potency for the stimulation of insulin release; *3*) this glucose dependency is lost in STZ-D rats; and *4*) this abnormality in diabetic rats was corrected only slightly by the normalization of the plasma glucose concentration by insulin treatment.

When we began this study, we intended to demonstrate the glucose dependency of stimulation of insulin release by GLP-I compared with that brought on by GIP. While this article was being prepared, Weir et al. (8) reported the glucose-dependent increase in insulin release by 1 nM GLP-I-(7–37) from the isolated perfused rat pancreas. This study is consistent with theirs in that no significant increase in IRI level was observed at an ambient glucose concentration of 2.8 mM. Komatsu et al. (9) reported that insulin release from the isolated perfused nondiabetic rat pancreas was significantly stimulated by 25 nM GLP-I at a perfusate glucose concentration 2.8 mM, which may be ascribed to the higher concentrations of GLP-I used but not a COOH-terminal difference in GLP-I. Our recent study (19) and that of Weir et al. (8) demonstrated that GLP-I-(7–36)-amide, GLP-I-(7–37), and GLP-I-(7–37)-amide have the same potency to stimulate insulin secretion.

The effects of GIP and GLP-I on glucagon release were also dependent on the ambient glucose concentration, although the effects of both hormones were completely opposite. Pederson and Brown (7) clearly demonstrated that the glucagonotropic action of GIP is negatively correlated with ambient glucose concentration. The inhibition of glucagon release by GLP-I has been demonstrated in isolated dog (13), rat (9), and pig (20) pancreas perfusions, and this is the first study that shows its glucose dependency. Similar negative dependency on the ambient glucose concentration has been demonstrated concerning the glucagon response to arginine in the isolated perfused rat pancreas (21,22).

In diabetic rats, these glucose-dependent effects on insulin and glucagon release by GIP and GLP-I were completely lost. The net increase in insulin release by GLP-I or GIP in diabetic rats was ~50% that of nondiabetic rats at 5.6 mM glucose (Fig. 5). In human in vivo studies, the response of β-cells to infusion of GIP was markedly reduced in hyperglycemic conditions in nonobese NIDDM subjects compared with nondiabetic subjects (12,23), and Nauck et al. (24) demonstrated a reduced incretin effect in NIDDM patients, notwithstanding the same increase in the plasma GIP level. As yet, there have been no previous reports concerning the effects of GLP-I on pancreatic hormone secretion in NIDDM. Our in vitro study confirmed the selectively reduced β-cell insensitivity to GIP and GLP-I because the same degree of β-cell response to arginine was demonstrated in the same type of diabetic rats at ambient glucose concentrations of 2.8 to ~16.7 mM (25; unpublished observations). These results suggest that a mechanism in signal transduction different from that for arginine operates for GIP or GLP-I stimulation of insulin release.

The glucose dependency of glucagon release by GLP-I

TABLE 1
Plasma glucose concentrations during insulin treatment of diabetic rats

Time	Fed plasma glucose level (mM)
0900	13.3 ± 1.4
1100	5.4 ± 0.5
1300	6.8 ± 0.8
1500	8.5 ± 0.9
1700	10.4 ± 1.4
1900	14.3 ± 1.6

Values are means ± SE (*n* = 23). Insulin (5 U/kg s.c.) was given at 0900.

and GIP was also impaired in diabetic rats. No significant inhibition by GLP-I and no significant stimulation by GIP were found in this experiment. There is no previous report on this. Concerning arginine, Leahy et al. (26) reported that the glucose regulation of arginine-stimulated glucagon secretion was intact in neonatal STZ-D rats, although the glucose reduction alone did not cause a glucagon response in the same type of diabetic rats. A normal glucagon response to arginine has also been shown in a group of patients with mild NIDDM (27). These results also suggest a difference in signal transduction in pancreatic α-cells between GIP or GLP-I and arginine.

The treatment of diabetic rats with insulin over a period of 6 days brought about a slight recovery in the glucose dependency of the insulin response to GLP-I, but the abnormal glucagon response was not ameliorated. The glucose dependency of the β-cell response to arginine showed greater recovery in the same type of experiment (26,28). The hyperresponse to arginine at 2.8 mM glucose in untreated STZ-D rats was normalized by 24 h of insulin treatment, although the response to 16.7 mM glucose alone was not restored (26). The arginine-induced insulin release from the isolated perfused pancreas of neonatal STZ-D rats improved with 5 days of insulin treatment (5 U · kg^{-1} body wt · day^{-1} s.c.; 28). The dose of insulin and the degree of improvement in plasma glucose by the insulin treatment in our experiment were similar to those of the latter study. Therefore, the poor improvement in the glucose dependency of insulin release in response to GLP-I by insulin treatment is not necessarily due to the incomplete normalization of the plasma glucose of diabetic rats in this study. The rats given STZ at birth seem to be an experimental model of a lean type of NIDDM because of their specific failure to release insulin in response to glucose (29,30). Therefore, these results suggest the difficulty in normalizing a reduced incretin effect in NIDDM by conventional treatment.

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