Effect of GIP and GLP-1 antagonists on insulin release in the rat

**Tseng, Chi-Chuan, Xiao-Ying Zhang, and M. Michael Wolfe.** Effect of GIP and GLP-1 antagonists on insulin release in the rat. Am. J. Physiol. 276 (Endocrinol. Metab. 39):E1049–E1054, 1999.—Glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) are potent insulinotropic peptides released from the small intestine. To examine their relative contribution to postprandial insulin release, a specific GIP antagonist (ANTGIP) and a GLP-1 antagonist, exendin-(9—39)-NH₂, were infused into rats after an intragastric glucose meal. In control rats, plasma glucose and insulin levels rose gradually during the first 20 min and then decreased. Exendin-(9—39)-NH₂ administration inhibited postprandial insulin secretion by 32% at 20 min and concomitantly increased plasma glucose concentrations. In contrast, ANTGIP treatment not only induced a 54% decrease in insulin secretion but also a 15% reduction in plasma glucose levels 20 min after the glucose meal. In vivo studies in rats demonstrated that glucose uptake in the upper small intestine was significantly inhibited by the ANTGIP, an effect that might account for the decrease in plasma glucose levels observed in ANTGIP-treated rats. When the two antagonists were administered to rats concomitantly, no potentiating effect on either insulin release or plasma glucose concentration was detected. Glucose meal-stimulated GLP-1 release was not affected by ANTGIP administration, whereas postprandial glucagon levels were diminished in rats receiving exendin-(9—39)-NH₂. The results of these studies suggest that GIP and GLP-1 may share a common mechanism in stimulating pancreatic insulin release. Furthermore, the GIP receptor appears to play a role in facilitating glucose uptake in the small intestine.

**Materials and Methods**

Oral glucose tolerance test. Sprague-Dawley rats (250–300 g) purchased from Charles River (Kingston, MA) were fasted overnight with access to water. Animals were administered glucose (1 g/kg) by oral gavage as a 40% solution (wt/vol). Blood samples were collected from the tail vein of conscious unrestrained rats into heparinized capillary tubes at time 0 and at 10, 20, 30, and 60 min after the glucose meal. ANTGIP (5 µg/250 g body wt), exendin-(9—39)-NH₂ (3 µg/250 g body wt, Bachem, Torrance, CA), or a combination of ANTGIP and exendin-(9—39)-NH₂ was given intraperitoneally at time 0 after baseline blood samples were obtained. The concentrations of ANTGIP and exendin-(9—39)-NH₂ were determined by performing a dose-response curve for each peptide and by selecting the lowest concentrations that exhibit maximal insulin inhibitory effect. Control animals were injected with the same volume of 0.9% NaCl. Blood samples were kept in ice-chilled tubes containing 250 kallikrein-inhibitor units of aprotinin and 0.1 mM dithiothreitol (Sigma) and centrifuged at 4°C for 30 min, and the plasma was stored at −20°C until analysis for glucose, insulin, or peptides. Glucose levels were determined with hexokinase and glucose-6-phosphate dehydrogenase (1). Insulin concentrations were measured with a radioimmunoassay kit (Rat Insulin RIA Kit, Linco Research, St. Charles, MO). Glucagon radioimmunoassays were performed with the double-antibody method (Diagnostic Products, Los Angeles, CA). Intracell assay variation was 15.7% with incubation of 35 pg of glucagon and 4.1% with 564 pg.
glucagon. Interassay variation was 15.7% with incubation of 37 pg glucagon and 5.7% with 534 pg glucagon. The lowest detection limit for glucagon was 13 pg/ml (3.7 pm). No significant cross-reactivity with any related peptide, such as GLP-1, GLP-2, GIP, vasoactive intestinal polypeptide, or secretin was noted. Plasma GLP-1 and GIP concentrations were measured with double-antibody method according to the protocol of the manufacturer (Peninsula Laboratories). The lowest detection limit for both GIP and GLP-(7–36)-NH2 was 10 pg/ml. The GLP-1 assay detected only amidated GLP-1, and no cross-reaction with other related peptides was identified.

D-Glucose absorption in vivo. D-Glucose absorption from jejunum was measured in vivo in the rat according to the method described by Hirsh et al. (14). Sprague-Dawley rats (250–300 g) were fasted with access to water for 24 h to minimize luminal contents during surgery. Rats were anesthetized with intraperitoneal urethane (Sigma, 1.25 g/kg body wt) and were maintained under anesthesia for the duration of the experiment. After laparotomy, a 35 cm segment of jejunum starting 5 cm distal to the ligament of Treitz was isolated, cleaned by gently flushing with 20 ml of warm 0.9% NaCl, and cannulated at both ends. Each segment was perfused twice, first as a control and second as a test (after ANTGIP administration) or a repeat control. The luminal perfusate, a Krebs-bicarbonate saline solution containing 5 mmol/l D-[14C]glucose (Amersham, Arlington Heights, IL), and 3H-labeled polyethylene glycol (PEG, NEN, Boston, MA) were used to correct for fluid movement into the extracellular space. The solution was heated to 37°C and gassed with 95% O2–5% CO2 to maintain the pH at 7.4. The perfusion system was single pass with a flow rate of 1.6 ml/min, with a Harvard PHD 2000 pump (Harvard Apparatus, South Natick, MA). The effluent from the luminal circuit was collected at 5-min intervals for up to 30 min. After the first experiment, the segment was cleaned by gently flushing with 20 ml of warm saline, at which time ANTGIP (20 µg/kg body wt) was injected subcutaneously before initiation of the second infusion. After the second perfusion, the jejunal loops were rinsed, ligated, and removed. The jejunal loop was then opened along the mesenteric border, the mucosal lining was scraped off with a glass slide, and the dry weight of each loop was determined. Radioactivity of all samples was determined with a liquid scintillation counter (LS 6500, Beckman, Fullerton, CA). Glucose and PEG absorption from the jejunum was calculated by changes in radioactivity of glucose and PEG in the affluent and effluent solutions and expressed as micromoles per minute per gram dry weight. Intramucosal accumulation of D-glucose in each animal was calculated after correction for extracellular substrate (amount of D-glucose absorbed amount of polyethylene glycol absorbed). The effect of ANTGIP on glucose absorption was determined by comparing mucosal D-glucose concentration between the first (no ANTGIP) and the second (with ANTGIP) experiment.

Statistics. Results are expressed as means ± SE. Statistical analysis was performed with ANOVA and Student’s t-test. P values < 0.05 were considered to be statistically significant.

RESULTS

Dose-response curves of ANTGIP and exendin-(9–39)-NH2 on insulin release. The effect of ANTGIP and exendin-(9–39)-NH2 on plasma insulin concentrations after a glucose meal was examined at 20 min when maximal inhibition occurred. ANTGIP and exendin-(9–39)-NH2 dose dependently inhibited glucose-induced insulin secretion with maximal inhibition achieved at 5 and 3 µg/250 g body wt, respectively (Fig. 1. A and B).

No further decrease in insulin levels was observed when higher doses of ANTGIP and exendin-(9–39)-NH2 were administered. Hence, these concentrations of ANTGIP (5 µg/250 g body wt) and exendin-(9–39)-NH2 (3 µg/250 g body wt) were chosen for the following oral glucose tolerance test studies.

Plasma glucose concentrations. After intragastric glucose administration, the mean plasma glucose levels in control rats increased from 82.5 ± 3.5 to 174.4 ± 3.6 mg/dl at 20 min and then plateaued (Fig. 2). The administration of exendin-(9–39)-NH2 produced a significant increase in plasma glucose concentration during the first 20 min (glucose concentration of 186.2 ± 2.4 mg/dl at 20 min). In contrast, rats receiving either ANTGIP alone or the combination of ANTGIP and exendin-(9–39)-NH2 exhibited lower glucose levels than control rats during the first 20 min, with concentrations of 148.7 ± 2.5 and 145.2 ± 4.0 mg/dl, respectively, at 20 min (Fig. 2; P < 0.05 compared with control). No differences in plasma glucose concentrations were detected between 30 and 60 min. As seen in Fig. 2, the plasma glucose concentrations of rats injected with ANTGIP or the combination of ANTGIP and exendin-(9–39)-NH2 were similar at all the time points examined.

Plasma insulin concentrations. In response to intragastric glucose administration, plasma insulin concen-
trations increased from a fasting level of 0.87 ± 0.16 to 2.75 ± 0.35 ng/ml at 20 min and then gradually decreased (Fig. 3). The administration of exendin-(9—39)-NH₂, ANTGIP, or the combination of exendin-(9—39)-NH₂ and ANTGIP significantly decreased plasma insulin levels by 32, 54, and 49%, respectively, at 20 min (insulin concentrations of 1.87 ± 0.20, 1.27 ± 0.40, and 1.38 ± 0.45 ng/ml, respectively, P < 0.05 compared with control). No significant differences were detected at 30 and 60 min after glucose administration (Fig. 3).

Plasma glucagon levels. Plasma glucagon concentrations were measured at 20 min, at which time the highest glucose levels were observed. In response to an oral glucose meal, plasma glucagon concentrations decreased from a fasting level of 181 ± 7 to 154 ± 5 pg/ml at 20 min (Fig. 4). ANTGIP administration did not affect postprandial glucagon levels significantly, whereas exendin-(9—39)-NH₂ injection significantly decreased glucagon levels to 138 ± 2 pg/ml at 20 min (P < 0.05, compared with control rats; Fig. 4). Plasma glucagon concentrations in rats receiving both exendin-(9—39)-NH₂ and ANTGIP did not differ from those of control rats.

Plasma GLP-1-(7—36)-NH₂ concentrations. Fasting plasma GLP-1-(7—36)-NH₂ levels were nearly identical in normal control and in ANTGIP-injected rats and ranged from 18 to 22 pg/ml (Fig. 5). In response to intragastric glucose infusion, plasma GLP-1-(7—36)-NH₂ levels in control rats increased significantly at 10 min and peaked at 30 min with concentration of 83 ± 5 pg/ml. GLP-1-(7—36)-NH₂ levels remained unaltered by the administration of ANTGIP throughout the entire experimental period (Fig. 5).

Effects of ANTGIP on D-glucose absorption in vivo. To examine whether ANTGIP affected glucose transport in the small intestine, D-glucose absorption from the rat
jejunum was measured in vivo. In control experiments during which both the first and second perfusions were performed in the absence of ANTGIP, d-glucose uptake was not significantly different (data not shown). In response to d-glucose infusion, plasma GIP level increased from 0.82 ± 0.25 ng/ml at basal state to 1.48 ± 0.13 ng/ml at the end of the first 30-min infusion (P < 0.05). When ANTGIP was administered before enteral glucose infusion reduced insulin release by 32 and 54% at 20 min, respectively. Together, these data suggest that approximately one-third of meal-stimulated insulin secretion is due to the incretin activities of GIP and GLP-1. Furthermore, because no additive effect was observed in animals receiving both exendin-(9—39)-NH₂ and ANTGIP when compared with either receptor antagonist alone, it would appear that the insulin-stimulatory properties of GIP and GLP-1 may be mediated, at least in part, through a common pathway. These results support recent observations by Pederson et al. (24), who demonstrated similar GIP- and GLP-1-mediated insulin secretion in wild-type and GLP-1-receptor knockout mice. Furthermore, enhanced GIP secretion and insulino depressive action in the GLP-1 receptor knockout mice are also consistent with the notion that GIP and GLP-1 may share an identical signaling pathway in the β-cell (24). Our current conclusions are, however, based on a glucose meal. Previous studies (3, 25) have shown that the release of GIP into the circulation after a meal depended on two major nutrient stimuli, carbohydrate and fat. Thomas et al. (26) reported that an amino acid mixture containing arginine, histidine, isoleucine, leucine, lysine, and threonine also provided a potent stimulus for GIP release. In contrast, few studies have examined nutrient-stimulated GLP-1 release. Nevertheless, oral glucose, arginine, or mixed meal administration has been demonstrated to enhance GLP-1 release (6, 10, 12). Further studies with other nutrients will be necessary to determine whether these effects observed in the present study are glucose specific.

Recently, several studies have shown that GIP stimulates GLP-1 release in rats (2, 13), as well as in isolated canine intestinal L cells (8). The authors of these studies proposed that GIP might exert only a "permissive action" on the β-cell via direct stimulation of GLP-1 secretion. Studies in human subjects have, however, failed to confirm these findings (20), and the present studies demonstrating that the GIP antagonist exhibited more profound inhibition of insulin secretion than the GLP-1 antagonist are also inconsistent with this conclusion. Our current study suggests that the incretin function of GIP may be mediated through both a direct effect on islet β-cells and the indirect stimulation of GLP-1 release. The latter effect does not appear to involve receptor-mediated processes, because postprandial GLP-1 secretion was not affected by the administration of GIP-receptor antagonist. In the current study, the fasting plasma amidated GLP-1 levels were compatible to those reported by Ørskov et al. (22). In response to a glucose meal, amidated GLP-1 rose significantly, but its concentrations were lower than those after a regular meal observed by Ørskov et al. (25 vs. 41 pm; Ref. 22). Although plasma glycine-extended GLP-1 and GLP-1-(7—37) levels are not measured in this study, it is unlikely that these peptides are relevant to our conclusions because the majority of circulated GLP-1 after meal is the amidated form (22). Whether GIP antagonist affects the release of nonamidated GLP-1 requires additional investigation.

The increase in postprandial plasma glucose levels after the administration of exendin-(9—39)-NH₂ is consistent with findings from previous reports (17, 29) and appears to account for the reduction in postpran-
dial glucagon release in exendin-treated rats. Previous reports have shown that GIP exhibited stimulatory effects on glucagon secretion in the rats, whereas GLP-1 inhibited glucagon release (11, 18). These effects were, however, shown to depend on the ambient glucose concentration (18). In our study, the plasma glucagon concentration in rats given both exendin-(9—39)-\(\text{NH}_2\) and ANTGIP was significantly higher than that of exendin-treated rats, consistent with above observations that plasma glucose concentration plays a more physiologically pertinent role than GLP-1 or GIP in modulating postprandial glucagon release. Although the precise mechanism for exendin-induced hyperglycemia has not been elucidated, this effect may be attributed to a reduction of the insulin response (17) or to a decrease in glucose utilization in peripheral tissues (7). Although not determined in the current study, Cheeseman and Tseng (4) have recently demonstrated that GLP-1 has no effect on \(\alpha\)-glucose uptake in the jejunum. In contrast to exendin, ANTGIP administration in the present studies significantly decreased both insulin and glucose levels after a glucose meal. The latter appears to be due, at least in part, to the inhibition of glucose uptake in the small intestine, as demonstrated in our in vivo studies.

The ability of the GIP-receptor antagonist to inhibit glucose uptake suggests that this function is receptor mediated, a notion further supported by the localization of the GIP receptor to the small intestinal mucosa by in situ hybridization (28). Moreover, our results are also consistent with the observations of Cheeseman and Tseng (4), who reported that the infusion of GIP, but not GLP-1, resulted in significant increases in carrier-mediated glucose uptake in the rat jejunum. In the current study, the concomitant administration of ANTGIP and exendin-(9—39)-\(\text{NH}_2\) reduced plasma glucose levels to those seen in ANTGIP-treated rats, which indicates that the GIP receptor plays an important role in regulating glucose transport in the upper small intestine. Further studies will be required to determine whether additional mechanisms, such as effects on peripheral glucose utilization, are involved in exerting the physiological properties attributed to GIP.

In summary, the current report demonstrates that both GIP and GLP-1 play an essential role in regulating postprandial insulin secretion and that their incretin effects account for \(\geq 50\%\) of insulin release after a glucose meal. Furthermore, the incretin properties of GIP and GLP-1 may be mediated, in part, through common mechanisms. Finally, as GIP antagonism inhibits glucose uptake in the small intestine, a similar approach may prove useful in improving postprandial hyperglycemia commonly seen in diabetic patients, as well as in treating disorders characterized by obesity.

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