Postprandial Stimulation of Insulin Release by Glucose-dependent Insulinotropic Polypeptide (GIP)

Effect of a Specific Glucose-dependent Insulinotropic Polypeptide Receptor Antagonist in the Rat

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Abstract

Glucose-dependent insulinotropic polypeptide (GIP) is a 42-amino acid peptide produced by K cells of the mammalian proximal small intestine and is a potent stimulant of insulin release in the presence of hyperglycemia. However, its relative physiological importance as a postprandial insulinotropic agent is unknown. Using LGIPR2 cells stably transfected with rat GIP receptor cDNA, GIP (1–42) stimulation of cyclic adenosine monophosphate (cAMP) production was inhibited in a concentration-dependent manner by GIP (7–30)-NH_2. Competition binding assays using stably transfected L293 cells demonstrated an IC_{50} for GIP receptor binding of 7 nmol/liter for GIP (1–42) and 200 nmol/liter for GIP (7–30)-NH_2, whereas glucagonlike peptide-1 (GLP-1) binding to its receptor on βTC3 cells was minimally displaced by GIP (7–30)-NH_2. In fasted anesthetized rats, GIP (1–42) stimulated insulin release in a concentration-dependent manner, an effect abolished by the concomitant intraperitoneal administration of GIP (7–30)-NH_2 (100 nmol/kg). In contrast, glucose-, GLP-1–, and arginine-stimulated insulin release were not affected by GIP (7–30)-NH_2. In separate experiments, GIP (7–30)-NH_2 (100 nmol/kg) reduced postprandial insulin release in conscious rats by 72%. It is concluded that GIP (7–30)-NH_2 is a GIP-specific receptor antagonist and that GIP plays a dominant role in mediating postprandial insulin release. (*J. Clin. Invest.* 1996. 98:2440–2445.) Key words: glucagonlike peptide-1 (7–36) (GLP-1) • incretin • insulin • GIP receptor

Introduction

Insulin release induced by the ingestion of glucose and other nutrients is due in part to both hormonal and neural factors (1). Several gastrointestinal regulatory peptides have been proposed as incretins (2–4), the substance(s) believed to mediate the enteroinsular axis and that may play a physiological role in maintaining glucose homeostasis. Among these candidates, only glucose-dependent insulinotropic polypeptide (GIP)[1] and glucagonlike peptide-1 (7–36) (GLP-1) appear to fulfill the requirements to be considered physiological stimulants of postprandial insulin release (5–8).

Although both GIP and GLP-1 possess significant insulinotropic properties, controversy exists regarding their relative physiological roles in stimulating insulin release. Some studies have demonstrated that GIP and GLP-1 are equally potent in their capacity to stimulate insulin release (9, 10), whereas others have suggested that GLP-1 possesses greater insulinotropic properties (11, 12). Recently, using a putative specific antagonist to the GLP-1 receptor, exendin (9–39), Wang et al. demonstrated that exendin reduced postprandial insulin release by 48% and thus concluded that GLP-1 might contribute substantially to postprandial stimulation of insulin secretion (13). More recent studies, however, have shown that exendin might also displace GIP binding from its receptor and thereby reduce GIP-stimulated cyclic adenosine monophosphate (cAMP) generation (14, 15). Therefore, the antagonist properties of exendin (9–39) might not be limited to GLP-1. The availability of a GIP-specific receptor antagonist would be invaluable for determining the precise roles of these peptides in mediating postprandial insulin secretion.

In the present report, using a reporter L cell line (LGIPR2) stably transfected with rat GIP receptor cDNA, we have identified a GIP fragment (GIP [7–30]-NH_2) as a specific GIP receptor antagonist. We first determined the inhibitory effect of this antagonist (referred to as ANTGIP) on GIP-stimulated cAMP production in vitro in LGIPR2 cells, followed by an examination of its binding properties in stably transfected L293 cells. The in vivo action of ANTGIP on glucose-, GLP-1–, GIP-, and arginine-induced insulin release was then examined in anesthetized rats. Finally, the effect of ANTGIP on postprandial insulin release was investigated in conscious rats.

Methods

Chemicals. Porcine GIP and GLP-1(7–36) were obtained from Peninsula Laboratories Inc. (Belmont, CA). Various peptide fragments of GIP, including GIP(21–30)-NH_2, GIP (16–30)-NH_2, GIP (7–30)-NH_2, GIP (1–30)-NH_2, and GIP (31–44), were synthesized at the Biopolymer Laboratory, Harvard Medical School, based on our pre-

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Received for publication 3 July 1996 and accepted in revised form 27 September 1996.

J. Clin. Invest.

0021-9738/96/12/2440/06 $2.00
Volume 98, Number 11, December 1996, 2440–2445

1. *Abbreviations used in this paper:* ANTGIP, GIP (7–30)-NH_2; GIP, glucose-dependent insulinotropic polypeptide; GLP, glucagonlike peptide; GLP-1, glucagonlike peptide-1.

viously published rat GIP cDNA sequence (16). Glucose and arginine were purchased from Sigma Chemical Co. (St. Louis, MO), and chlorophenol red–β-D-galactopyranoside was obtained from Boehringer Mannheim Biochemicals (Indianapolis, IN).

Rat GIP receptor cell line. To examine cAMP production in response to GIP and other peptide fragments, we utilized a cAMP reporter L cell line (LGIPR2) stably expressing rat GIP receptor cDNA, as described previously (17). LGIPR2 cells are stably transfected with a cAMP-dependent promoter from the VIP gene fused to the bacterial *lac Z* gene. When intracellular cAMP increases within these cells, *lac Z* gene transcription is activated, resulting in the accumulation of its product, β-galactosidase. The measurement of β-galactosidase in this system provided a convenient, inexpensive, and nonradioactive method for detecting changes in the levels of intracellular cAMP.

LGIPR2 cells were grown in DME containing 4.5 g/liter of glucose and 10% FCS. For each assay, 10^5 cells/well were seeded onto 24-well plates. After incubation overnight, peptides were added in various concentrations to the wells in the absence of 3-isobutyl-1-methylxanthine (IBMX) for 4 h, at which time maximal stimulation of β-galactosidase was determined. The medium was then removed and wells were rinsed once with PBS. The plates were then blotted briefly and frozen overnight at −70°C, and, after the addition of chlorophenol red-β-D-galactopyranoside, accumulated β-galactosidase was detected using a colorimetric assay, as described previously (17).

Binding studies. GLP(7–37) and porcine GIP (5 μg each) were iodinated by the chloramine-T method and were purified using C-18 cartridges (model Sep-Pak; Millipore Corp., Waters Chromatography, Milford, MA) using an acetonitrile gradient of 30–45%. The specific activity of radiolabeled peptides was 10–50 μCi/mg (18, 19). Aliquots were lyophilized and reconstituted in assay buffer at 4°C to a concentration of 3×10^5 cpm/100 μl. Binding studies were performed in desegregated stably transfected L293 or βTC3 cells, the latter a generous gift from Dr. S. Efrat (Diabetes Center, Albert Einstein College of Medicine, NY). The βTC3 cell line originally arose in a lineage of transgenic mice expressing an insulin-promoted, SV40 T-antigen hybrid oncogene in pancreatic β cells (20) and has previously been demonstrated to be responsive to both GIP and GLP (19). The receptor binding buffer contained 138 mM NaCl, 5.6 mM KCl, 1.2 mM $MgCl_2$, 2.6 mM $CaCl_2$, 10 mM Hepes, 10 mM glucose, and 1% BSA (fraction V, protease free, Sigma Chemical Co.). For binding assays, L293 (GIP binding) or βTC3 (GLP-1 binding) cells were cultured in DME containing 4.5 g/liter of glucose and 10% fetal bovine serum until 70% confluent. Cells were washed once with PBS and then harvested with PBS-EDTA solution. βTC3 cells were then suspended in assay buffer at a density of 2×10^6 cells/ml, and L293 cells were used at a density of 2.5×10^5 cells/ml. Binding was performed at room temperature in the presence of 3×10^5 cpm/ml of ^{125}I-GIP and -GLP. Nonsaturable binding was determined by the amount of radioactivity associated with cells when incubated in the presence of 10^{-6} M GIP, GLP, or 10^{-4} M ANTGIP. Specific binding was defined as the difference between counts in the absence and presence of unlabeled peptide.

Intravenous infusion of peptides in fasting anesthetized rats. Adult male Sprague-Dawley rats (250–350 g) were purchased from Charles River Laboratories (Kingston, MA). For infusion studies, rats were fasted overnight and then anesthetized using intraperitoneal sodium pentobarbital. The right jugular vein was cannulated with silicon polymer tubing (0.025 in. ID, 0.047 in. OD, Dow Corning Corp., Midland, MI), as described by Xu and Melethil (21). The tubing was then connected to an infusion pump (Harvard Apparatus Co., Inc., Millis, MA), and freshly made 0.9% NaCl, 5% glucose, arginine, GIP, or GLP-1 (peptides and arginine dissolved in 0.9% NaCl) was infused at a rate of 0.1 ml/min. Blood (0.5 ml each) was obtained at 0, 10, 20, and 30 min by translumbar vena cava puncture, as described by Winsett et al. (22), and samples were centrifuged at 2,000 *g* for 10 min. Serum samples were separated and stored at −20°C until assayed for insulin using a radioimmunoassay kit (ICN Biochemicals Inc., Costa Mesa, CA), and glucose, using a glucose meter (model One Touch II; Lifescan, Inc., Milpitas, CA).

Insulinotropic effect of GIP in trained conscious fed rats. Previous reports have indicated that the stress response to injection in untrained rats might alter their feeding and subsequently glucose and insulin levels (13). To avoid such a response, rats were trained for 10 d before experimentation. They were fasted from 1700 to 0800 h, and 0.9% NaCl (0.3 ml) was injected subcutaneously at 0800 before feeding. After the injection of 0.9% NaCl, animals were given rat chow for 30 min, after which it was removed. At the end of 10 d, the rats were accustomed to the injection and ate quickly (consuming 4–6 g of rat chow within 30 min). After fasting from 1700 the night before, rats were injected subcutaneously at 0800 with 0.3 ml of either 0.9% NaCl or ANTGIP (100 nmol/kg). This dose was chosen to approximate the amount of peptide used in the above anesthetized animal studies. After injection, six of the fasted control rats were killed to obtain baseline serum glucose and insulin levels. ANTGIP- or 0.9% NaCl-treated rats ($n = 6$ in each group) were offered chow for 30 min, after which food was withdrawn. Rats were then anesthetized by intraperitoneal sodium pentobarbital, and blood was collected by translumbar vena cava puncture at 20 and 40 min for the subsequent measurement of plasma insulin, glucose, and GLP-1.

Statistical analysis. All results are expressed as mean±SEM. Statistical analysis was performed using ANOVA and Student's *t* test. $P < 0.05$ was considered to be statistically significant.

Results

Effects of various peptide fragments on cAMP production in LGIPR2 cells. To define the biologically active region of the GIP and to explore the possibility of identifying a GIP-specific antagonist, we examined the effects of several peptide fragments of GIP on stimulating cAMP-dependent β-galactosidase production in LGIPR2 cells. Preliminary studies using LGIPR2 cells demonstrated that GIP(1–42) stimulated β-galactosidase production in a concentration-dependent manner, with the maximum effect observed at 4 h with 10^{-8} M (data not shown). As demonstrated in Fig. 1, 10^{-8} M GIP (1–30)-NH_2 stimulated β-galactosidase production to a similar degree, while none of the other peptide fragments tested, including

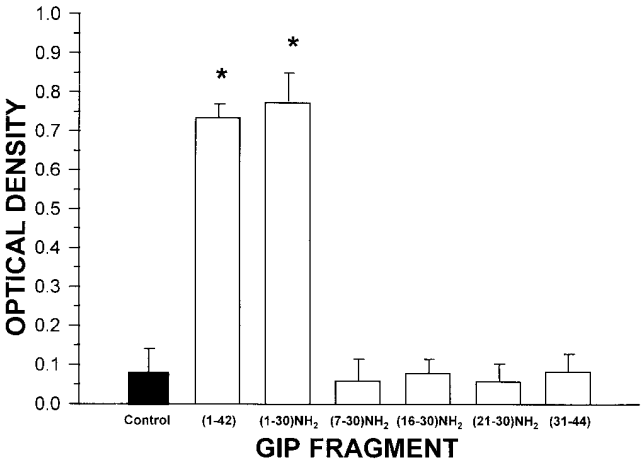

Figure 1. Cyclic AMP-dependent β-galactosidase generation in LGIPR2 cells in response to incubation with different fragments of GIP. LGIPR2 cells were incubated in the presence of 10^{-8} M GIP or different GIP fragments for 4 h, and β-galactosidase was measured as described in Methods and expressed in optical density (OD) units. Values are expressed as the mean±SE of quadruplicate measurements. *$P < 0.01$, compared to control.

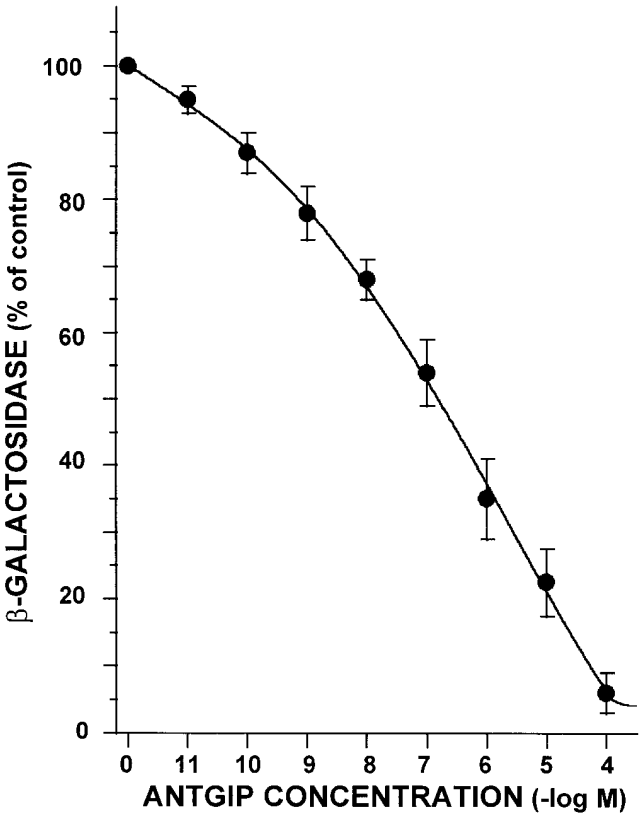

Figure 2. Dose-dependent inhibition of ANTGIP on GIP-induced cAMP-dependent β-galactosidase production in LGIPR2 cells. LGIPR2 cells were cultured in the presence of 10^{-8} M GIP and various concentrations of ANTGIP, as depicted on the horizontal axis. Values are expressed as the mean±SE of quadruplicate measurements.

GIP (7–30)-NH_2, GIP (16–30)-NH_2, GIP (21–30)-NH_2, and GIP (31–44), stimulated β-galactosidase generation above control levels. Furthermore, no changes in cAMP-dependent β-galactosidase levels were detected when LGIPR2 cells were incubated in the presence of higher concentrations of the smaller peptide fragments.

To examine whether any of these fragments might serve as an antagonist to GIP, LGIPR2 cells were incubated with 10^{-8} M GIP (1–42) and one of the peptide fragments at two different concentrations (10^{-8} M or 10^{-6} M) for 4 h. Only GIP (7–30)-NH_2 (ANTGIP) was found to attenuate the cAMP stimulatory effects exhibited by GIP (1–42); the inhibition was concentration-dependent, with half maximal inhibition occurring at 10^{-7} M (Fig. 2).

Receptor binding studies. Binding studies were performed in either L293 or βTC3 cells to determine the relative affinities of GIP, ANTGIP, and GLP-1 for both GIP and GLP-1 receptors. GIP and ANTGIP displaced the binding of ^{125}I-GIP to L293 cells in a concentration-dependent manner (Fig. 3), with an IC_{50} of 7 nM for GIP (n = 5) and 200 nM for ANTGIP (n = 4). Binding of ^{125}I-GLP–1 to its βTC3 cell receptor was displaced fully by GLP-1, but negligibly by ANTGIP, with an IC_{50} of 4 nM and 80 mM, respectively (n = 7; Fig. 3).

Fasted anesthetized rats. To examine the insulinotropic effect of GIP in vivo, fasted anesthetized rats were perfused continuously with three different concentrations of GIP (0.5, 1.0,

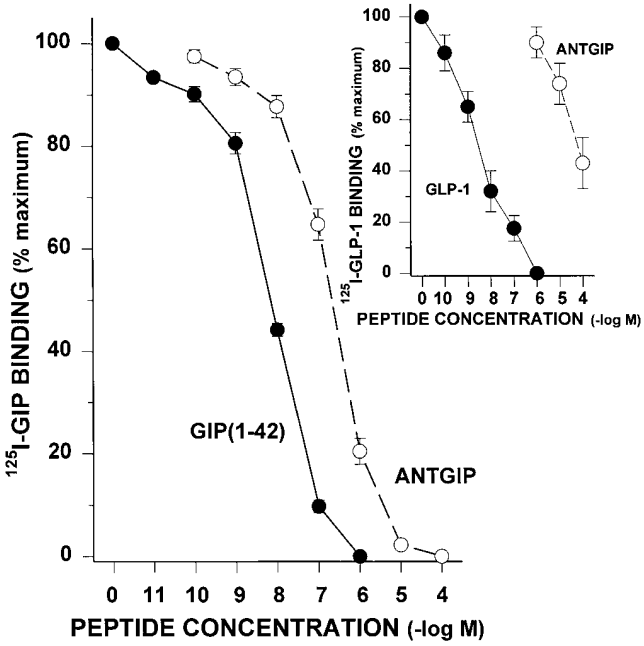

Figure 3. Competition of ^{125}I-GIP and ^{125}I-GLP-1 (*inset*) binding by GIP, GLP-1, and ANTGIP. GIP binding was examined using L293 cells, and GLP-1 binding was assessed using βTC3 cells. Values are expressed as a percentage of maximum specific binding and are the mean±SE, with assays performed in duplicate.

and 1.5 nmol/kg) at a rate of 0.1 ml/min for 30 min (10^{-8} M equivalent to 1 nmol/kg per 30 min). Significant increases in plasma insulin levels were first detected at 15 min, and after completion of the GIP infusion, insulin levels were elevated with all three GIP concentrations (43.5±2.7, 61.6±4.2, and 72.4±3.5 μIU/ml, respectively) compared to control (32.2±3.3 μIU/m, $P < 0.05$, Fig. 4). The concomitant administration of ANTGIP (100 nmol/kg) completely abolished the insulinotro-

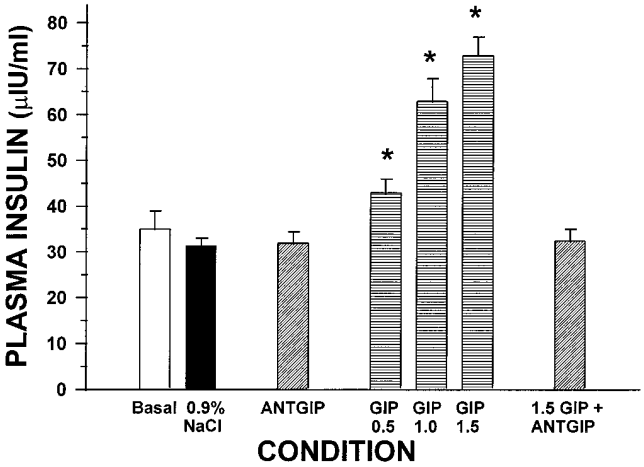

Figure 4. Plasma insulin concentrations (±SE) in fasted anesthetized rats after 30 min of GIP, ANTGIP, or 0.9 NaCl infusion. GIP was infused at 0.5, 1.0, and 1.5 nmol/kg, with the largest insulin stimulatory response seen with 1.5 nmol/kg. ANTGIP (100 nmol/kg) administered concomitantly with GIP 1.5 nmol/kg completely abolished its insulinotropic effect, whereas ANTGIP and 0.9% NaCl infusion had no effect on insulin secretion (n = 6 for each group). *$P < 0.05$, compared with basal levels.

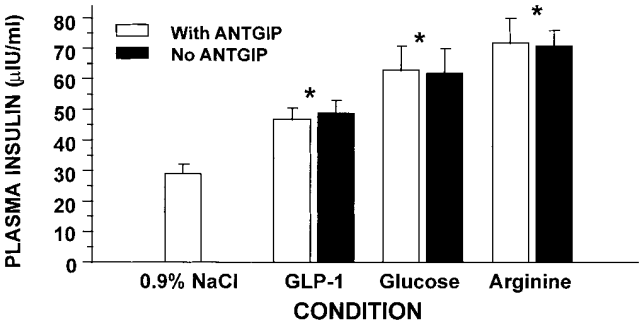

Figure 5. Plasma insulin concentrations (±SE) in fasted anesthetized rats after a 30-min infusion of GLP-1 (0.4 nmol/kg), glucose (0.8 g/kg), or arginine (375 mg/kg) with (*open bars*) or without (*solid bars*) ANTGIP (100 nmol/kg) ($n = 6$ for each group). *$P < 0.05$, compared with basal levels.

pic properties of GIP (1.5 nmol/kg), with plasma insulin returning to control values (Fig. 4).

To examine whether ANTGIP exerted a nonspecific effect on β cell function, GLP-1 (0.4 nmol/kg), glucose (0.8 g/kg), or arginine (375 mg/kg) was infused, in the presence or absence of the antagonist for 30 min, as described by Wang et al. (13). GLP-1, glucose, and arginine alone each significantly increased insulin levels after 15 min of infusion, and by 30 min, the insulin levels in GLP-1-, glucose-, and arginine-infused rats were 50.3±3.7, 63.1±2.5, 69.7±5.8 μIU/ml respectively ($P <$ 0.01, compared with control rats, 29.1±2.9 μIU/ml, Fig. 5). No significant change in the insulin response was detected when ANTGIP was administered concomitantly (Fig. 5).

Conscious trained fed rats. In response to consuming chow, serum glucose and plasma insulin levels increased significantly, with insulin levels of 38.7±5.3 and 58.9±3.7 μIU/ml at 20 and 40 min, respectively ($P < 0.05$, Fig. 6 *A*). These increases in

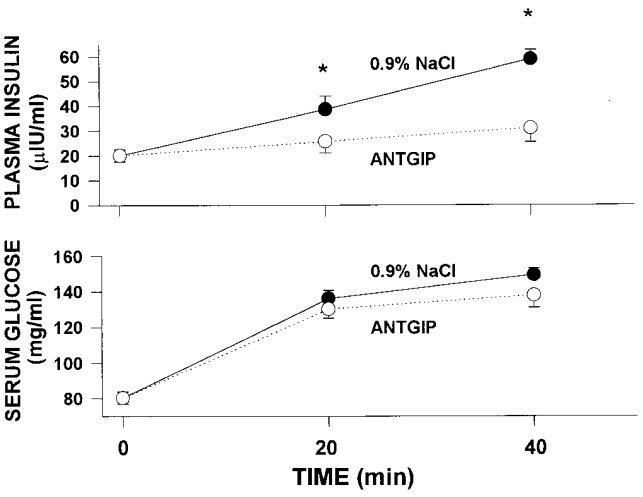

Figure 6. Postprandial plasma insulin and serum glucose levels (±SE) in conscious trained rats. Before feeding, all rats were injected with 0.9% NaCl subcutaneously for 10 d, as described in the Methods section. On the day of the experiment, ANTGIP (100 nmol/kg) or 0.9% NaCl (control) was injected subcutaneously before the meal was administered ($n = 6$ in each group). *$P < 0.01$ compared to ANTGIP injection.

plasma insulin level were nearly abolished by ANTGIP pretreatment; at 20 and 40 min, the plasma insulin concentrations were 25.3±4.7 and 27.1±2.6 μIU/ml, respectively ($P < 0.01$). Postprandial serum glucose concentrations were similar in both saline- and ANTGIP-treated rats (Fig. 6 *B*). To determine whether the effects of the GIP receptor antagonist were mediated through changes in GLP-1 release into the circulation, postprandial serum GLP-1 levels were measured in both control and ANTGIP-treated animals. Meal-stimulated serum GLP-1 concentrations were not affected by ANTGIP administration. After the ingestion of rat chow, serum GLP-1 levels at 20 min were 280±20 and 290±10 pg/ml in control and ANTGIP-treated rats, respectively; at 40 min, serum GLP-1 concentrations were 320±10 and 330±20 pg/ml, respectively.

Discussion

The results of this study demonstrate that GIP (7–30)-NH_2 is a specific GIP receptor antagonist. In LGIPR2 cells, ANTGIP inhibited the cAMP response to GIP in a concentration-dependent manner, and in L293 cells, the antagonist displaced GIP binding from its receptor. Furthermore, ANTGIP completely abolished the insulinotropic properties of GIP in fasted anesthetized rats, while not affecting GLP-1-, glucose-, or arginine-stimulated insulin release indicating that this antagonist is GIP specific. ANTGIP alone demonstrated no stimulatory effect on insulin release or cAMP generation in either intact rats or LGIPR2 cells, indicating the absence of any agonist properties. Although it is feasible that ANTGIP might exhibit agonist effects at higher concentrations, initial studies demonstrated that even at a concentration as high as 10^{-4} M, no increase in cAMP-dependent β-galactosidase level was detected in LGIPR2 cells.

The successful synthesis of a specific GIP receptor antagonist greatly facilitates investigation of the relative contribution of GIP in mediating the enteroinsular axis. As stated above, insulin release induced by the ingestion of glucose and other nutrients is due in part to both hormonal and neural factors (1). Although a number of gastrointestinal regulatory peptides have been proposed as putative incretins, GIP and GLP-1 are the most likely physiological insulinotropic peptides. After oral glucose administration, serum GIP levels increase several fold (23–27), and although the increment in plasma GLP-1 concentration in response to glucose is also significant, it is far smaller in magnitude (27–30). In human volunteers, Nauck et al. (31) showed that GIP was a major contributor to the incretin effect after oral glucose, whereas GLP-1 appeared to play a minor role. Shuster et al. also suggested that GIP was the most important, but not the sole, mediator of the incretin effect in humans (30). Using a GLP-1 receptor antagonist exendin (9–39), Wang et al. detected a 50% decrease in postprandial insulin secretion in exendin-treated rats (13). Administration of exendin also reduced 70% of insulin release following intraduodenal glucose infusion (32). Recent studies, however, have demonstrated that exendin not only displaces GIP binding from its receptor, but also inhibits cAMP generation in response to GIP stimulation (14, 15). Therefore, the antagonist properties of exendin do not appear to be GLP-1 specific. In the present report, we have shown that in response to the administration of a GIP-specific receptor antagonist—ANTGIP—to rats, a 72% decrease in postprandial insulin release was observed. ANTGIP did affect GLP-1 binding to its recep-

tor, and the insulinotropic effect of GLP-1 is preserved in vivo in the presence of ANTGIP. Furthermore, postprandial GLP-1 levels were not affected by ANTGIP. These findings are consistent with a dominant role for GIP in mediating the enteroinsular axis.

The finding of similar plasma glucose levels in both control and ANTGIP-treated fed rats is not surprising. Wang et al. (13) demonstrated an approximate 50% reduction in postprandial insulin levels in exendin-treated rats, whereas plasma glucose levels increased minimally from 7.5 to 8.7 mmol/liter. The physiological significance of this minor increment in glucose level is not clear. In this report, we found that serum glucose concentrations remained largely unchanged despite a marked decrease in serum insulin levels in ANTGIP-treated rats. Although not evaluated in the present study, the results of the present study are consistent with the notion that insulin is not the sole mediator of glucose homeostasis, whose maintenance is dependent on numerous neurohumoral factors. These factors include hormones, such as pancreatic glucagon, cortisol, and growth hormone, and physiological events, including peripheral and hepatic glucose uptake.

In this study, we have also confirmed previous studies (33) indicating that GIP (1–30)-NH_2 might be one of the biologically active forms of mature GIP. As shown in Fig. 1, GIP (1–30)-NH_2 was nearly equipotent to GIP (1–42) in stimulating cAMP-dependent β-galactosidase production in LGIPR2 cells. These findings are consistent with the observations of Wheeler et al. (14), who reported that both GIP(1–42) and GIP(1–30) exhibited similar stimulatory properties for cAMP production in COS-7 cell transiently expressing GIP receptor cDNA. Moreover, Kieffer et al. (19) found that GIP (1–30) competitively inhibited binding of GIP (1–42) to the GIP receptor in βTC3 cells. These data suggest the possibility of cellular processing of GIP (1–42) to yield biologically active α-amidated GIP (1–30). Further investigation will obviously be required to establish its natural presence in the small intestinal mucosa and the circulation.

In conclusion, the results of the present studies demonstrate that GIP (7–30)-NH_2 is a specific receptor antagonist of naturally occurring GIP. GIP (7–30)-NH_2 inhibits GIP-induced cAMP generation and insulin release, but does not affect the insulinotropic effects of other secretagogues such as glucose, arginine, and GLP-1. Furthermore, circulating insulin levels decreased by 72% in response to the concomitant administration of GIP (7–30)-NH_2 to chow-fed rats, indicating that GIP plays a dominant role in mediating postprandial insulin secretion.

Acknowledgments

This study was supported, in part, by National Institutes of Health grants KO8DK-08753 (C.C. Tseng) and RO1DK-48042 (M.M. Wolfe). T.J. Kieffer is a recipient of a Howard Hughes Institute Fellowship.

References

1. Creutzfeldt, W., and R. Ebert. 1985. New developments in the incretin concept today. *Diabetologia.* 28:565–573.

2. Unger, R.H., and A.M. Eisentraut. 1969. Entero-insular axis. *Arch. Intern. Med.* 123:261–266.

3. Ebert, R., and W. Creutzfeldt. 1987. Gastrointestinal peptides and insulin secretion. *Diab. Metab. Rev.* 3:1–16.

4. Dupré, J. 1991. The Endocrine Pancreas. Raven Press, New York. 253 pp.

5. Dupré, J., S.A. Ross, D. Watson, and J.C. Brown. 1973. Stimulation of insulin secretion by gastric inhibitory polypeptide in man. *J. Clin. Endocrinol. Metab.* 37:826–828.

6. Nauck, M., W. Schmidt, J. Ebert, J. Strietzel, P. Cantor, G. Hoffman, and W. Creutzfeldt. 1989. Insulinotropic properties of synthetic human gastric inhibitory peptide in man: interaction with glucose, phenylalanine, and cholecystokinin 8. *J. Clin. Endocrinol. Metab.* 69:654–662.

7. Kreymann, B., G. Williams., M.A. Ghatei, and S.R. Bloom. 1987. Glucagon-like peptide-1 7–36: a physiological incretin in man. *Lancet.* ii:1300–1304.

8. Mojsov, S., G.C. Weir, and J.F. Habener. 1987 Insulinotropin: glucagon-like peptide 1 (7–37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. *J. Clin. Invest.* 79:616–619.

9. Schmid, R., V. Schusdziarra, R. Aulehner, N. Weigert, and M. Classen. 1990. Comparison of GLP-1 and GIP on release of somatostatin-like immunoreactivity and insulin from the isolated rat pancreas. *Z. Gastroenterol.* 28: 280–284.

10. Suzuki, S., K. Kawai, S. Ohashi, H. Mukai, Y. Murayana, and K. Yamashita. 1990. Reduced insulinotropic effects of glucagon-like peptide I-amide and GIP in isolated perfused diabetic rat pancreas. *Diabetes.* 39:1320–1325.

11. Siegel, E.G., A. Schulze, W.E. Schmidt, and W. Creutzfeldt. 1992. Comparison of the effect of GIP and GLP-1 on insulin release from rat pancreatic islets. *Eur. J. Clin. Invest.* 22:154–157.

12. Shima, K., M. Hirota, and C. Ohboshi. 1988. Effect of GLP-1 on insulin secretion. *Regul. Pept.* 22:245–252.

13. Wang, Z., R.M. Wang, A.A. Owji, D.M. Smith, M.A. Ghatei, and S.R. Bloom. 1995. Glucagon-like peptide-1 is a physiological incretin in rat. *J. Clin. Invest.* 95:417–421.

14. Wheeler, M.B., R.W. Gelling, C.H.S. McIntosh, J. Georgiou, J.C. Brown, and R.A. Pederson. 1995. Functional expression of the rat pancreatic islet glucose-dependent insulinotropic polypeptide receptor: ligand binding and intracellular signaling properties. *Endocrinology.* 136:4629–4639.

15. Gremlich, S., A. Porret, E.H. Hani, D. Cherif, N. Vionnet, P. Froguel, and B. Thorens. 1995. Cloning, functional expression, and chromosomal localization of the human pancreatic islet glucose-dependent insulinotropic polypeptide receptor. *Diabetes.* 44:1202–1208.

16. Tseng, C.-C., L.A. Jarboe, S.B. Landau, E.K. Williams, and M.M. Wolfe. 1993. Glucose-dependent insulinotropic peptide: Structure of the precursor and tissue-specific expression in rat. *Proc. Natl. Acad. Sci. USA.* 90:1992–1996.

17. Usdin, T.B., E. Mezey, D.C. Button, M.J. Brownstein, and T.I. Bonner. 1993. GIP receptor, a member of the secretin-vasoactive intestinal peptide receptor family, is widely distributed in peripheral organs and the brain. *Endocrinology.* 133:2861–2870.

18. Hunter, M.W., and F.C. Greenwood. 1962. Preparation of iodine-131 labelled human growth hormone of high specific activity. *Nature (Lond.).* 194: 495–498.

19. Kieffer, T.J., B. Verchere, C.D. Fell, Z. Huang, J.C. Brown, and R.A. Pederson. 1993. Glucose-dependent insulinotropic polypeptide stimulated insulin release from as tumor-derived β-cell line. *Can. J. Physiol. Pharmacol.* 71: 917–922.

20. Efrat, S., S. Linde, H. Kofod, D. Spector, M. Delannoy, S. Grant, D. Hanahan, and S. Baekkeskov. 1988. β-cell lines derived from transgenic mice expressing a hybrid insulin gene-oncogen. *Proc. Natl. Acad. Sci. USA.* 85:9037–9041.

21. Xu, Z.-X., and S. Melethil. 1990. Simultaneous sampling of blood, bile, and urine in rats for pharmacokinetic studies. *J. Pharmacol. Method.* 24:203–208.

22. Winsett, O.E., C.M. Townsend, and J.C. Thompson. 1985. Rapid and repeated blood sampling in the conscious laboratory rat: a new technique. *Am. J. Physiol.* 249:G145–146.

23. Cleator, I.G.M., and R.H. Gourlay. 1975. Release of immunoreactive gastric inhibitory polypeptide by oral ingestion of food substances. *Am. J. Surg.* 130:128–135.

24. Nauck, M.A., E. Homberger, E.g., Siegel, R.C. Allen, R.P. Eaton, R. Ebert, and W. Creutzfeldt. 1986. Incretin effects of increasing glucose loads in man calculated from venous insulin and C-peptide response. *J. Clin. Endocrinol. Metab.* 63:492–498.

25. Nauck, M., F. Stockmann, R. Ebert, and W. Creutzfeldt. 1986. Reduced incretin effect in type 2 diabetes. *Diabetologia.* 29:46–52.

26. Salera, M., P. Giacomoni, L. Pironi, C. Ustra, M. Capelli, A. Georgi, M. Miglioli, and L. Barbara. 1983. Circadian rhythm of gastric inhibitory polypeptide in man. *Metabolism.* 32:21–24.

27. Kreymann, B., M.A. Ghatei, G. Williams, and S.R. Bloom. 1987. Glucagon-like peptide 1 7-36: a physiological incretin in man. *Lancet.* ii:1300–1304.

28. Ørskov, C., and J.J. Holst. 1987. Radioimmunoassays for glucagon-like peptides 1 and 2. Scand. *J. Clin. Lab. Invest.* 47:165–174.

29. Ørskov, C., J.U. Jeppesen, S. Madsbad, and J.J. Holst. 1991. Proglucagon products in plasma of noninsulin-dependent diabetics and nondiabetic controls in the fasting state and after oral glucose and intravenous arginine. *J. Clin. Invest.* 87:415–423.

30. Shuster, L.T., V.L.W. Go, R.A. Rizza, P.C. O'Brien, and F.J. Service. 1988. Potential incretins. *Mayo Clin. Proc.* 63:794–800.

31. Nauck, M.A., E. Bartels, C. Ørskov, R. Ebert, and W. Creutzfeldt. 1993. Additive insulinotropic effects of exogenous synthetic human gastric inhibitory polypeptide and GLP-1 (7–36) amide infused at near-physiological insulinotropic hormone and glucose concentrations. *J. Clin. Endocrinol. Metab.* 76:912–917.

32. Kolligs, F., H.C. Fehmann, R. Göke, and B. Göke. 1995. Reduction of the incretin effect in rats by the GLP-1 receptor antagonist exendin. *Diabetes.* 44:16–19.

33. Rossowski, W.J., S. Zacharia, Z. Mungan, V. Ozmen, A. Ertan, L.M. Baylor, N.Y. Jiang, and D.H. Coy. 1992. Reduced gastric acid inhibitory effect of a pGIP(1–30)-NH_2 fragment with potent pancreatic amylase inhibitory activity. *Regul. Pep.* 39:9–17.