The Expression and Function of Glucose-Dependent Insulinotherectic Polypeptide in the Embryonic Mouse Pancreas

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OBJECTIVE—Glucose-dependent insulinotherectic polypeptide (GIP) is a member of a structurally related group of hormones that also includes glucagon, glucagon-like peptides, and secretin. GIP is an incretin, known to modulate glucose-induced insulin secretion. Recent studies have shown that glucagon is necessary for early insulin-positive differentiation, and a similar role for incretins in regulating embryonic insulin-positive differentiation seems probable. Here we studied the role of GIP signaling in insulin-positive differentiation in the embryonic mouse pancreas.

RESEARCH DESIGN AND METHODS—The ontogeny of the GIP ligand and GIP receptor in the embryonic pancreas was investigated by immunohistochemistry and RT-PCR. GIP signaling was inhibited in cultured embryonic pancreata using morpholine-ring antisense against GIP ligand and receptor, or small interfering RNA (siRNA) for GIP ligand and receptor. Markers of endocrine cells and their progenitors were studied by immunohistochemistry and RT-PCR.

RESULTS—GIP and GIP receptor mRNA were both detected in the embryonic pancreas by embryonic day 9.5 and then persisted throughout gestation. GIP was generally coexpressed with glucagon by immunostaining. The GIP receptor was typically coexpressed with insulin. Morpholine-ring antisense or siRNA against either GIP ligand or GIP receptor both inhibited the differentiation of insulin-positive cells. Inhibition of GIP or its receptor also led to a decrease in the number of Pdx-1–positive and sox9-positive cells in the cultured embryonic pancreas. The number of Pax6- and Nkx2.2-positive cells, representative of developing pancreatic endocrine cells and β-cells, respectively, was also decreased.

CONCLUSIONS—GIP signaling may play a role in early embryonic pancreas differentiation to form insulin-positive cells or β-cells. Diabetes 60:548–554, 2011

Glucose-dependent insulinotherectic polypeptide (GIP) is an incretin. Incretins are hormones released from the gut in response to nutrient ingestion that potentiate glucose-stimulated insulin secretion (1). GIP and glucagon-like peptide 1 (GLP-1) are the two main incretins, identified as mediators of the process by which administration of oral glucose provokes a greater stimulation of insulin release than does an intravenous glucose challenge (2). This connection between gut and the pancreatic islets has been termed the “enteroinsular axis” (3) and appears to be responsible for 50% of postprandial insulin release (2,4). GIP is released from enteroendocrine K-cells in the duodenum, primarily in response to the ingestion of glucose or fat, and potentiates insulin secretion in a glucose-dependent manner (5). A recent study now reports the presence of a bioactive form of GIP in the α-cells that promotes insulin secretion in the adult islet β-cells (6). GLP-1 is a proglucagon-derived peptide hormone that is synthesized and secreted by the enteroendocrine L-cells in the distal ileum and the colon. Preproglucagon can be alternatively processed to produce glicentin or oxyntomodulin and GLP-1 (7).

The incretin GLP-1 can enhance β-cell growth and differentiation. GLP-1 receptor null mice, however, do not show a loss of β-cell development. Interestingly, these mice were found to have upregulated GIP release and GIP-induced insulin release (8). On the other hand, the GIP receptor (GIPR) null mice, which also did not show any obvious β-cell defect, showed enhanced sensitivity to GLP-1, suggesting that there may be important cooperation between these two incretin signaling pathways (9).

And we others previously demonstrated that glucagon signaling to the glucagon receptor is necessary for early insulin-positive differentiation (10,11). This glucagon dependency also was suggested by the observation that Pax6 or prohormone convertase 2 null mice, which both lack glucagon-positive cells, also lack early insulin-positive differentiation (12,13). Although it was reported that GIP is produced in enteroendocrine K-cells in the duodenum of the adult, GIP was also found to be produced in the human fetal pancreas by 18 weeks’ gestation (14). The function of this GIP in the developing pancreas remains to be elucidated. Here we show that GIP is located in the embryonic mouse pancreas, and loss-of-function studies in vitro suggest that insulin differentiation in the embryonic pancreas depends on GIP signaling.

RESEARCH DESIGN AND METHODS—Isolation of embryonic pancreas. All animal experiments were performed in accordance with the guidelines for animal experimentation of the Children’s Hospital of Pittsburgh, Pennsylvania. Male and female CD-1 mice were mated overnight. The presence of a vaginal plug the next morning was indicative of pregnancy, and noon of that day was designated as embryonic day 0.5 (E0.5). Embryonic pancreata were harvested on various embryonic days from E9.5 to E18.5 using microdissection techniques as described previously (15).

Pancreas organ culture conditions. E11.5 pancreata with surrounding mesenchyme were grown using the hanging drop method by placing them in a standard 35-mm dish with Dulbecco’s modified Eagle’s medium F-12 (Gibco BRL, Grand Island, NY) containing 10% FBS and 1% antibiotic/antimycotic solution (10,000 units/mL penicillin G, 10,000 units/mL streptomycin sulfate, and 200 µg/mL amphotericin B [Gibco BRL]). Each drop contains 40 µl media.
starting with a 10-min denaturation step at 95°C, followed by 40 cycles of denaturation at 95°C for 30 s, 58°C for 40 s for primer annealing, and 72°C for 45 s for extension.

**Semiquantitative PCR.** Real-time semiquantitative PCR was carried out with a SyberGreen PCR Master Mix in an iCycler IQ apparatus (Bio-Rad, Hercules, CA). The amplification conditions were as follows: 94°C for 5 min, followed by 40 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s. A mouse β-tubulin primer set was used to normalize the amount of total cDNA for each gene expression experiment. Serial concentrations of cDNA (107, 106, 105, 104, 103, and 102 molecules/μL) were used for the calibration of a standard curve for the given primer. The PCR products were separated and visualized on the agarose gel. At least five different samples were analyzed at each sequential age to quantify the target gene. Primer sequences used are listed as forward then reverse, 5′ to 3′: β-tubulin, 5′-CGTGGGCCCAGATCCTCACG-3′ and 5′-ACCACTACTGCTCCCTG-3′, which amplify a product of 102 bp; GIP, 5′-GACTTCGATTCCATGATA-3′ and 5′-TTGCACTCCTTTGCTCCTT-3′, which amplify a product of 238 bp; and GIPR, 5′-CTCATCCTATCCGACAT-3′ and 5′-GGAAACCTCCTGGAAGGAACTT-3′, which amplify a product of 226 bp.

**RESULTS**

**GIP and GIPR ontology in the developing pancreas.**

The ontology of GIP ligand and GIPR in the developing pancreas was investigated by immunohistochemistry at serial gestational days. GIP-positive staining could be identified as early as E9.5 and continued through E18.5 (Fig. 1A, C, and E; Supplementary Fig. 1A, E, I, and M). Pdx1-positive cells (Supplementary Fig. 1B, F, J, and N) were present in pancreatic epithelium at early stages and in β-cells at later stages. Neither of these two Pdx1–positive cell populations stained positive for GIP (Supplementary Fig. 1D, H, L, and P). Glucagon-positive staining and merged images (Supplementary Fig. 1C, G, K, O, and D, H, L, P) revealed that GIP ligand appeared to colocalize with glucagon, but not with Pdx1 or insulin (insulin data not shown). RT-PCR for GIP ligand showed that mRNA expression of GIP also was present as early as E9.5 and continued through later gestational stages and into the postnatal period (Fig. 2A).
Semiquantitative RT-PCR showed a gradually increasing GIP mRNA level beginning at E9.5 (Fig. 2C). In situ hybridization was unable to detect GIP ligand, perhaps because of a low expression level (data not shown). GIP is a member of a family of structurally related hormones that includes glucagon and glucagon-like peptides. Thus, to check for immunostaining cross-reactivity, we preincubated GIP, GLP-1, or glucagon peptide (100 μg/mL) with GIP antibody or glucagon antibody before immunohistochemistry of embryonic mouse pancreas. GIP immunostaining was not influenced by preincubation with glucagon peptide or GLP-1 peptide, but was appropriately blocked by GIP peptide (Supplementary Fig. 2). Similarly, glucagon immunostaining was not influenced by preincubation with GIP peptide or GLP-1 peptide, but was blocked appropriately by glucagon peptide (Supplementary Fig. 2). Thus, our GIP antibody does not appear to have any significant cross-reactivity with GLP-1 peptide or glucagon peptide. However, Fujita et al. (6) used a similar antibody against GIP and found cross-reactivity between GIP and glucagon. Thus, a member of a family of structurally related hormones including glucagon and glucagon-like peptides. Therefore, a low expression level (data not shown). GIP is a member of a family of structurally related hormones that includes glucagon and glucagon-like peptides. Thus, to check for immunostaining cross-reactivity, we preincubated GIP, GLP-1, or glucagon peptide (100 μg/mL) with GIP antibody or glucagon antibody before immunohistochemistry of embryonic mouse pancreas. GIP immunostaining was not influenced by preincubation with glucagon peptide or GLP-1 peptide, but was appropriately blocked by GIP peptide (Supplementary Fig. 2). Similarly, glucagon immunostaining was not influenced by preincubation with GIP peptide or GLP-1 peptide, but was blocked appropriately by glucagon peptide (Supplementary Fig. 2). 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Next we inhibited GIP signaling by morpholine-ring antisense as an alternative to siRNA. Pancreases cultured for 6 days in the presence of 40 μmol/L morpholine-ring antisense against GIP showed greatly suppressed GIP expression when compared with Morpholino control antisense-treated tissues (Supplementary Figs. 5A and 5B and 6A). GIP Morpholino antisense at 40 μmol/L significantly (P < 0.05) suppressed insulin-positive differentiation expression when compared with Morpholino control antisense-treated tissues (Supplementary Figs. 5A and 5B and 6A). GIP Morpholino antisense at 40 μmol/L significantly (P < 0.05) suppressed insulin-positive differentiation

![Image](image-url)

**FIG. 3.** Immunohistochemical staining for GIPR at serial gestational ages E11.5 (A–D), E13.5 (E–H), E15.5 (I–L), and E18.5 (M–P). Definite GIPR-positive staining was first identified at E13.5 and continued through E18.5 (A, E, I, and M). Insulin (B, F, J, and N), glucagon (C, G, K, and O), and merged images (D, H, L, and P) are shown. GIPR expression overlapped primarily with insulin. At E13.5, a few glucagon cells also expressed GIPR (compare 3D and 3H) in addition to the insulin-positive cells that expressed GIPR (magnification size bars: D and H = 20 μmol/L; L and P = 50 μmol/L). (A high-quality digital representation of this figure is available in the online issue.)

![Image](image-url)

**FIG. 4.** Immunohistochemistry of GIP siRNA-treated embryonic pancreas showed decreased insulin and Pdx-1–positive staining (A, B, E, and F). There was no apparent change in amylase staining (C and G). Area of insulin staining was calculated by using image analysis software, and the positive proportion of the entire pancreas was then calculated. The insulin-staining area of GIP siRNA-treated cultured pancreas was significantly less than the GAPDH siRNA-treated cultured pancreas (n = 3, P = 0.003) (I). Pdx-1–positive cells were also significantly decreased in GIP siRNA-treated pancreas (J). (A high-quality digital representation of this figure is available in the online issue.)
in response to nutrient ingestion, especially glucose or fat synthesized and secreted from intestinal K-cells located mainly in the duodenum and proximal jejunum. GIP is secreted in response to nutrient ingestion, especially glucose or fat

![Fig. 5. Semiquantitative RT-PCR was performed for insulin and glucagon (A and B, respectively) and showed significant decreases of mRNA with GIP siRNA. Similarly, the Pax6- and Nkx2.2-positive staining areas were decreased significantly in GIP siRNA-treated pancreas (C and D). Error bars represent SEM.](image)

(Fig. 6A and B; Supplementary Fig. 6B). Similar to siRNA inhibition, GIP Morpholino antisense treatment also resulted in a significant decrease in Pdx-1 (Fig. 6A and B) and Nkx2.2 expression (Fig. 6D and E). Sox9-positive cells were also decreased in GIP ligand antisense-treated samples (Fig. 6J and K). Interestingly, we did not see any increase in cleaved caspase staining in GIP ligand antisense-treated samples, suggesting cells were not undergoing apoptosis (Fig. 6D and E). We did not detect any significant change in the number of BrdU-positive cells between control and GIP antisense-treated samples (Fig. 6G and H).

**Blocking of GIPR expression in explant cultures in vitro led to decreased insulin-positive differentiation.** A significant decrease in insulin-positive differentiation was found in embryonic pancreas treated with GIPR morpholino-ring antisense (Fig. 6C; Supplementary Fig. 5D and E). The insulin-positive area was significantly decreased, compared with control-treated pancreas (Supplementary Fig. 7A). Semiquantitative RT-PCR for insulin and glucagon mRNA also showed a significant reduction after 40 μmol/L GIPR morpholino-ring antisense treatment (Supplementary Fig. 7B and C). Pdx-1 and Sox9 (markers of progenitor cells) were also reduced in GIP-receptor antisense-treated pancreas (Fig. 6C and L). Similar to GIP ligand antisense, here again after blocking GIP the receptor, we did not see any significant change in apoptosis (Fig. 6F) or cell proliferation (Fig. 6I).

Similarly, immunohistochemistry after GIPR siRNA treatment (instead of Morpholino treatment) of cultured pancreas showed reduced insulin (Fig. 7A–C) and Pdx-1 (Fig. 7A, B, and D). Semiquantitative RT-PCR for GIPR mRNA showed a significant reduction of GIPR mRNA after GIPR siRNA treatment (Fig. 7E), confirming effective knockdown of expression by the siRNA. Immunohistochemistry of GIPR siRNA-treated pancreas also showed a significant decrease in Pax6- (P = 0.001) and Nkx2.2-positive (P = 0.0008) cell numbers (Supplementary Fig. 8).

**DISCUSSION**

**GIP and GIPR ontogeny.** In mature animals, GIP is synthesized and secreted from intestinal K-cells located mainly in the duodenum and proximal jejunum. GIP is secreted in response to nutrient ingestion, especially glucose or fat (17). GIPR is a member of the seven transmembrane-spanning, heterotrimeric G-protein-coupled receptor superfamily. In the adult pancreas, GIPR expression can be seen in the islets (18). However, relatively little is known about the ontogeny of GIP and GIPR. In our study, GIP mRNA and protein was found in the developing embryonic pancreas. The specificity of the antibody used in this study was confirmed by peptide preincubation with exogenous peptides GLP-1, GIP, or glucagon before immunohistochemistry. Our experiments confirmed that GIP is localized to the developing pancreas, especially in glucagon-positive cells. Interestingly, beginning at E13.5, GIPR was localized to insulin-positive cells, consistent with its known presence in β-cells in the adult (18). There was then a transition from early GIP expression before E15.5, when most GIPR-positive cells were glucagon positive/insulin negative, to later in gestation, when nearly all GIPR-positive cells were insulin positive/glucagon negative. These results suggest a possible paracrine-signaling pathway from glucagon-positive cells to insulin progenitor cells. Such signaling from glucagon cells to induce insulin cell formation is supported by studies in which removal of glucagon or glucagon receptor attenuates early insulin cell differentiation (10,11,13). Tseng et al. (19) were unable to detect GIP mRNA in the pancreas of late-gestation fetal rats. Late gestational pancreas is rife with RNases, and their lack of detection of what presumably is a low mRNA copy number for GIP may explain this discrepancy.

**GIP signal inhibition in early pancreatic development.** GIPR knockout mice (20,21) did not appear to have pancreatic abnormalities that would suggest a developmental defect. We specifically studied the embryonic pancreas of GIPR null mutant mice (a gift from Dr. S. Seino, Osaka, Japan), which also appeared to be normal (data not shown). However, these mice are known to exhibit mild glucose intolerance in response to an oral glucose challenge. It is possible that this glucose intolerance phenotype, as well as a possible embryonic phenotype in GIPR null mutant mice, is dampened because of compensation from other signaling pathways, such as GLP-1 signaling. GIPR null mutant mice have been shown to have enhanced GLP-1 receptor sensitivity (21). However, GIPR/GLP1-R double null mutant mice are also born normal, so further alternative pathways may be in play in the double mutant
mice. The glucose intolerance in adult GIPR null mutant mice appeared to be due to reduced levels of glucose-stimulated insulin secretion. Interestingly, GIPR knockout mice had a normal glucose curve after an intraperitoneal glucose challenge, suggesting that enteral glucose, with stimulation of intestinal (K-cell) GIP release, was bypassed when the glucose was given intraperitoneally.

In our study, siRNA and morpholine-ring antisense against GIP or GIPR were used to inhibit GIP signaling in the developing pancreas in vitro. Inhibition of GIP by these methods resulted in a decrease in insulin-positive differentiation, as well as a decrease in Pdx-1-, Pax6-, and Nkx2.2-positive cells. Interestingly, we did not see an increase in sox9-positive cells, as we saw in a previous study where inhibition of ngn-3–mediated endocrine differentiation resulted in a significant increase in sox9- and DBA-positive progenitor cells (22). In addition, we did not see any obvious change in proliferation or cell death after GIP or GIPR antisense treatment. Thus, the lack of endocrine differentiation in these antisense-treated cultures could represent a failure to differentiate. We have described previously that inhibition of ngn-3 expression in the embryonic pancreas led to a significant increase in DBA-positive ductal cells, with several of those cells coexpressing sox-9, suggesting that they may represent expansion of an pancreatic epithelial progenitor pool (22). Similarly, we also saw an increase in duct cells in the embryonic pancreas when we inhibited glucagon signaling using Morpholino antisense (K.P., C.S., P.G., G.K.G., unpublished data). Similar effects on an epithelial progenitor cell population may occur after GIP signaling inhibition.

The discrepancy between our in vitro results and findings from genetically altered mice may be due to the sudden inhibition of GIP signaling that occurs with our in vitro systems, without the chance for an adaptive response to compensate for the loss of GIP signaling that may occur in null mutant mice. This possibility could be tested with conditional incretin receptor mutants. Counter-intuitively, GIPR knockout mice have been shown to develop an increase in \( \beta \)-cell area in the adult, perhaps in response to the oral glucose intolerance mentioned above.

Paracrine GIP signaling from \( \alpha \)-cells to \( \beta \)-cells. We and others previously demonstrated that glucagon is necessary for the early development of insulin-positive cells in the pancreas because of paracrine actions of glucagon (10,11). Similarly, our results imply that GIP signaling to the GIPR is also necessary for early \( \beta \)-cell development. Such a role for GIP ligand signaling to its receptor is also suggested by the embryonic localization of GIP and GIPR in adjacent glucagon- and insulin-positive cells, respectively.
K.P. and M.K. researched data. K.P. wrote the manuscript. S.T., C.S., N.L., J.P., P.G., and Y.E.-G. contributed to discussion and review. G.K.G. contributed to discussion and reviewed and edited the manuscript. M.M. and S.S. contributed to discussion and review.

REFERENCES

1.Creutzfeldt W. The incretin concept today. Diabetologia 1979;16:75–85
2.Perley MJ, Kipnis DM. Plasma insulin responses to oral and intravenous glucose: studies in normal and diabetic subjects. J Clin Invest 1967;46:1954–1962
3.Unger RH, Eisentraut AM. Entero-insular axis. Arch Intern Med 1969;123:261–266
4.Efendic S, Portwood N. Overview of incretin hormones. Horm Metab Res 2004;36:742–746
5.Yip RG, Wolfe MM. GIP biology and fat metabolism. Life Sci 2000;66:91–103
6.Fujita Y, Wideman RD, Asadi A, et al. Glucose-dependent insulinotropic polypeptide is expressed in pancreatic islet alpha-cells and promotes insulin secretion. Gastroenterology 2010;138:1966–1975
7.Drucker DJ. Minireview: the glucagon-like peptides. Endocrinology 2001;142:521–527
8.Pederson RA, Satkunamarajah M, McIntosh CH, et al. Enhanced glucose-dependent insulinotropic polypeptide secretion and insulinogetic action in glucagon-like peptide 1 receptor –/– mice. Diabetes 1998;47:1046–1052
9.Pamir N, Lynn FC, Buchan AM, et al. Glucose-dependent insulinotropic polypeptide receptor null mice exhibit compensatory changes in the enteroinsular axis. Am J Physiol Endocrinol Metab 2003;284:E931–E939
10.Prasadan K, Daume E, Preuett B, et al. Glucagon is required for early insulin-positive differentiation in the developing mouse pancreas. Diabetes 2002;51:3229–3236
11.Vuguin PM, Kedees MH, Cui L, et al. Ablation of the glucagon receptor gene increases fetal lethality and produces alterations in islet development and maturation. Endocrinology 2006;147:3905–4006
12.Dohrmann C, Gruss P, Lemaire L. Pax genes and the differentiation of neuroendocrine hormone-producing endocrine cells in the pancreas. Mech Dev 2000;92:47–54
13.Vincent M, Guz Y, Rozenberg M, et al. Abrogation of protein convertase 2 activity results in delayed islet cell differentiation and maturation, increased alpha-cell proliferation, and islet neogenesis. Endocrinology 2003;144:4061–4069
14.Portela-Gomes GM, Johansson H, Oiding L, Grimelius L. Co-localization of neuroendocrine hormones in the human fetal pancreas. Eur J Endocrinol 1999;141:526–533
15.Gittes GK, Galante PE, Hanahan D, Rutter WJ, Debske HT. Lineage-specific morphogenesis in the developing pancreas: role of mesenchymal factors. Development 1996;122:439–447
16.Sakai T, Larsen M, Yamada KM. Fibronectin requirement in branching morphogenesis. Nature 2003;423:876–881
17.Baggio LL, Drucker DJ. Biology of incretins: GLP-1 and GIP. Gastroenterology 2007;132:2131–2157
18.Usdin TB, Mezey E, Button DC, Brownstein MJ, Bonner TI. Gastric inhibitory polypeptide receptor, a member of the secretin-vasoactive intestinal peptide receptor family, is widely distributed in peripheral organs and the brain. Endocrinology 1993;133:2861–2870
19.Tseng CC, Boylan MO, Jarboe LA, Williams EK, Wolfe MM. Glucose-dependent insulinotropic peptide (GIP) gene expression in the rat salivary gland. Mol Cell Endocrinol 1995;115:13–19
20.Miyawaki K, Yamada Y, Ban N, et al. Inhibition of gastric inhibitory polypeptide signaling prevents obesity. Nat Med 2002;8:738–742
21.Miyawaki K, Yamada Y, Yano H, et al. Glucose intolerance caused by a defect in the enteroinusular axis: a study in gastric inhibitory polypeptide receptor knockout mice. Proc Natl Acad Sci U S A 1999;96:14843–14847
22.Prasadan K, Tulachan S, Guo P, Shiota C, Shah S, Gittes G. Endocrine-committed progenitor cells retain their differentiation potential in the absence of neurogenin-3 expression. Biochem Biophys Res Commun 2010;396:1036–1041
23.Kieffer TJ, McIntosh CH, Pederson RA. Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 in vitro and in vivo by dipeptidyl peptidase IV. Endocrinology 1995;136:3585–3596
24.Gauld VA, Parker JC, Harriott P, Flatt PR, O’Harte FP. Evidence that the major degradation product of glucose-dependent insulinotropic polypeptide, GIP(3-42), is a GIP receptor antagonist in vivo. J Endocrinol 2002;175:525–533
25.Mentlein R. Dipeptidyl-peptidase IV (CD26): role in the inactivation of regulatory peptides. Regul Pept 1999;85:9–24