

Alternative form of glucose-dependent insulinotropic polypeptide and its physiology

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ABSTRACT

Glucose-dependent insulinotropic polypeptide (GIP) was first extracted from porcine gut mucosa and identified as “incretin” decades ago. Though early studies have shown the possible GIP isoforms by gel filtration profiles from porcine or human intestinal extracts analyzed by radioimmunoassay (RIA), GIP is currently believed to consist of 42 amino acids (GIP1-42), which are released from gut K-cells and promote postprandial insulin release. In fact, GIP1-42 is usually processed from proGIP by the action of prohormone convertase (PC) 1/3 in the gut. GIP expression is occasionally found in the intestinal glucagon-like peptide-1-secreting cells, suggesting gene expression of both GIP and proglucagon can co-exist in identical cells. However, GIP1-42 immunoreactivity is rarely found in α -cells or other pancreatic endocrine cells of wild-type mammals. Interestingly, we found that short-form GIP1-30 is expressed in and released from pancreatic α -cells and a subset of enteroendocrine cells through proGIP processing by PC2. GIP1-30 is also insulinotropic and modulates glucose-stimulated insulin secretion in a paracrine manner. It is also suggested that short-form GIP1-30 possibly plays a crucial role for the islet development. It has not been well elucidated whether expression of GIP1-30 is modulated in the diabetic status, and whether GIP1-30 might have therapeutic potentials. Our preliminary data suggest that short-form GIP1-30 might play important roles in glucose metabolism.

HISTORICAL ASPECTS

The early studies in the 1960s showed that the intestinal mucosa extract inhibited acid secretion in the dog stomach, which was designated “enterogastrone.” Then, “gastric inhibitory polypeptide (GIP)” was purified from porcine mucosal extracts and based on gastric acid inhibitory activity^{1,2}. Dupré *et al.*³ successfully showed that intravenous GIP injection could enhance insulin secretion during intravenous glucose challenge in humans. Several studies showed that GIP was released by oral glucose loading or food absorption^{4,5}, and that GIP also potentiated insulin secretion in a glucose-dependent manner⁶. Thereafter, GIP was spelled out as “glucose-dependent insulinotropic polypeptide” reflecting its physiological insulinotropic activity, and was recognized as an “incretin.” The sequence of purified GIP peptide was determined and identified as consisting of 42 highly conserved amino acids among porcine, bovine and

humans^{7,8}. GIP1-42 was designated GIP5000 (natural GIP), as its molecular weight is 4983.6. Whereas another larger molecule, GIP8000 (or 8kD GIP), has been identified from porcine and human extracts from proximal jejunum, analyzed by RIA after fractionation through gel filtration with different GIP antisera^{9,10}. GIP8000 was proposed to be a precursor product of the GIP gene product. However, further analysis has not been examined to investigate molecular characteristics of GIP8000.

In addition to gut mucosa, several early studies showed that GIP immunoreactivity was detectable in mammalian pancreatic extracts through RIA. Interestingly, a study showed that the pancreas was the major source of circulating GIP in Burmese pythons.¹¹ In that study, both plasma GIP and glucagon levels were sixfold increased by re-feeding at 1 day after starvation. GIP and glucagon were distributed more largely in the pancreas compared with the intestine or the stomach in the pythons. Pancreatic distribution of GIP remarkably decreased with re-feeding, suggesting that GIP was released from the pancreas as a part of digestive responses in the pythons, and concurrently increased the plasma concentration with glucagon.

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IS GIP EXPRESSED IN PANCREATIC α -CELLS?

As some preliminary studies in the past showed that GIP immunoreactivity through RIA had been detectable in pancreatic extracts, several studies tried to elucidate whether the pancreatic tissue was actually releasing GIP, and to find where GIP was expressed in the pancreas. A study showed that glucagon-secreting α -cells in rat pancreatic islets also contained a "GIP-like material," but the authors could not exclude the possibility that a GIP-like peptide was absorbed from the circulation¹². Another study examined various species including humans by immunohistochemistry, and showed that GIP immunoreactivity could be expressed not only in the gut, but also in the islet α -cells¹³. The GIP antiserum used in that study was believed not to cross-react with either pancreatic glucagon or enteroglucagon. Interestingly, many cells showing concomitant GIP and glucagon immunoreactivity were observed in the surgical specimens from patients with pancreatic glucagonomas¹³. However, a separate study concluded that GIP was not present in the pancreas, stomach and large intestine, but was released from enteroendocrine cells of the duodenum and jejunum¹⁴. Furthermore, the authors indicated *not* GIP *but* glicentin-like immunoreactivity was found in pancreatic α -cells. In these early studies, we need to consider limitations, as characterization or specificity of GIP antisera or antibodies might be insufficient. These GIP antisera or antibodies were polyclonal from rabbits or guinea pigs and raised against the whole GIP molecule. In addition, the amino (N-) terminus of GIP and pro-glucagon gene products including pancreatic glucagon, glucagon-like peptide (GLP-) 1, GLP-2, glicentin and oxyntomodulin are well conserved in many species with higher homology. Therefore, it was very difficult to exclude the possibility that GIP antisera or antibodies might cross-react with some parts of the proglucagon gene products.

Meanwhile, Buchan *et al.*¹⁵ developed a mouse monoclonal antibody (3.65H) specifically against the carboxyl (C-) terminus of GIP (GIP1-42) and compared with conventional rabbit and guinea-pig antisera to GIP by immunochemical analysis. The analysis using this monoclonal antibody showed that C-terminal immunoreactivity of GIP1-42 was not observed in the mammalian pancreas, but abundantly found in the gut endocrine cells. As results from the study were definitely conclusive, few studies have been subsequently carried out, investigating GIP distribution elsewhere except the gastrointestinal tract.

PROGIP IS PROCESSED BY PROHORMONE CONVERTASE 1/3 IN THE GUT K-CELLS

In humans, the *GIP* gene is located on chromosome 17. The *GIP* gene is translated to pre-proGIP (GIP precursor) messenger ribonucleic acid (mRNA) with approximately 800 base pairs, and its transcript consists of 153 amino acids including a signal peptide of 21 residues¹⁶. Ugleholdt *et al.*¹⁷ investigated how proGIP was processed in the gut K-cells. They showed that GIP was co-expressed with prohormone convertase (PC) 1/3 in the intestinal sections from wild-type mice, but never

with PC2, when the identical monoclonal antibody¹⁵ (3.65H) against C-terminal GIP1-42 was used for detection of GIP. PC1/3 null mice were largely impaired in processing to GIP1-42, whereas PC2 null mice presented normal processing of GIP1-42, analyzed by gel filtration of the gut extracts through RIA. Collectively, they concluded that PC1/3 was essential and sufficient for proGIP processing to GIP1-42, whereas PC2 did not play a role in GIP production at least in the gut K-cells. In contrast, the authors investigated proGIP processing *in vitro* using PC2-transfected GH4 cells and α -cell line α -TC1.9 cells. They interestingly pointed out the possibility that PC2 was able to cleave ProGIP to "other GIP fragments," which were not usually present in the gut extracts¹⁷.

GIP AND GLP-1, ARE THEY HOUSEMATES IN AN ENDOCRINE CELL?

It has been well recognized that GLP-1 and PYY are concomitantly expressed in identical colon L-cells. Nowadays, a lot of knowledge has been accumulated that many endocrine cells in the gastrointestinal tract can secrete two or more hormones, but anatomical distribution or combination of hormones are supposed to be destined by localization in the cephalo-caudal axis¹⁸.

Proglucagon and proGIP mRNA expression was identified in laser-captured single duodenal endocrine cells by reverse transcription polymerase chain reaction in human biopsied samples¹⁹. In addition, immunoreactivity of GLP-1 and GIP was co-expressed with glucokinase in the single gut cells (L/K or K/L-cells). Interestingly, the proportion of K/L cells in the duodenum was significantly increased in newly diagnosed type 2 diabetic patients¹⁹. In our study²⁰, double incretin-positive cells expressed both transcription factors Pdx1 and Pax6, whereas GLP-1 single positive cells expressed Pax6, but not Pdx1, in the rat ileum (Figure 1). The reporter assay showed that Pax6 could activate both GIP and proglucagon promoters, but Pdx-1 was able to enhance only the GIP promoter, when the 2.9-kb human GIP promoter or the 2.4-kb rat proglucagon promoter was examined. Therefore, Pax6 is the common key transcription for the two genes²⁰. Reimann *et al.*²¹ developed novel transgenic mice expressing Venus fluorescent protein under the proglucagon promoter. They sorted isolated cells from the gut of transgenic mice by flow cytometry, and examined the expression of GIP immunoreactivity by enzyme-linked immunosorbent assay. It was shown that PYY was observed in the proglucagon-positive (Venus-positive) cells only from the colon, but GIP was occasionally expressed in Venus-positive gut cells from the upper intestine. Collectively, GIP and GLP-1 might be in part housemates in a gut endocrine cell.

GIP1-30 EXPRESSION IN PANCREATIC α -CELLS AND A SUBSET OF ENTEROENDOCRINE CELLS

Similar to the double-incretin positive "gut K/L-cells" expressing both GIP and proglucagon genes^{19,20}, it is postulated that GIP might be coexpressed with glucagon in pancreatic α -cells. In

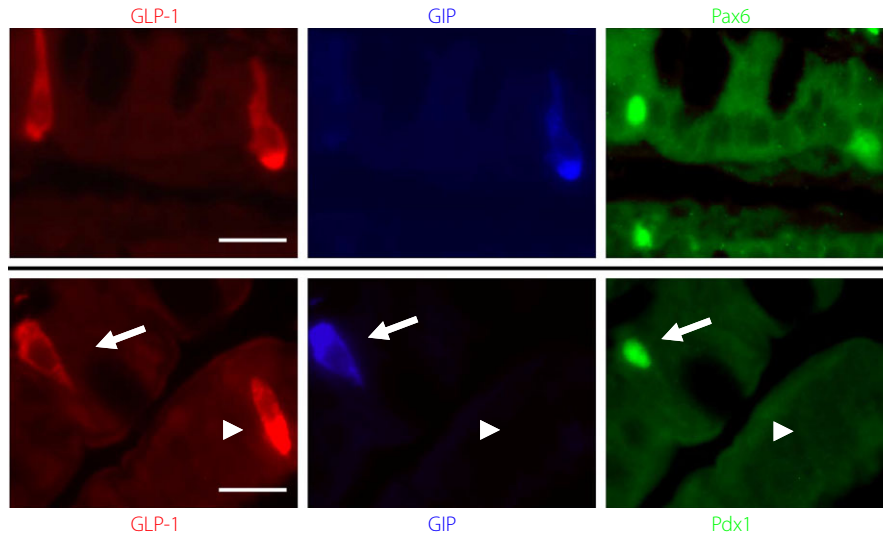


Figure 1 | The gut K/L cells express both glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). Immunohistochemistry of rat ileum. GLP-1 (red), GIP (blue) and transcription factors, Pax6 or Pdx-1 (green). Arrow shows a K/L cell expressing both GLP-1 and GIP, while arrowhead shows an L cell only expressing GLP-1. Adapted from Fujita *et al.*²⁰ with permission (magnification: $\times 400$).

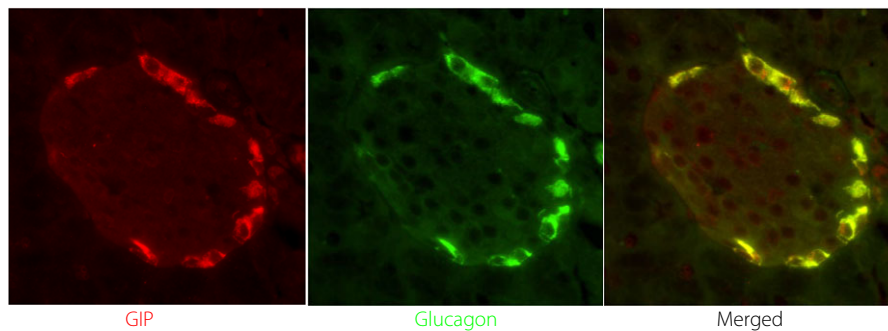


Figure 2 | Glucose-dependent insulinotropic polypeptide (GIP)1-30 is co-expressed in the pancreatic α -cells. Immunohistochemistry of a wild-type mouse islet. GIP (red), glucagon (green). GIP antibody against the midportion of GIP was used for detection^{22,23} (magnification: $\times 400$).

fact, GIP mRNA expression was examined by reverse transcription polymerase chain reaction and *in situ* hybridization, and present in the mouse islets²². Immunohistological analysis showed that GIP was localized in islet α -cells of mice, humans and pythons, using an antibody recognizing the midportion of GIP (Figure 2). However, GIP immunoreactivity was detected in islets not from wild-type mice, but from PC2 null mice, when the C-terminal GIP antibody¹⁵ (3.65H) was used for immunostaining (Figure 3), suggesting that GIP in the pancreas might be processed by PC2. Though ProGIP is typically cleaved by PC1/3 into GIP1-42 in the gut as discussed¹⁷, there is a putative site ³⁰KGKK (mouse ³⁰RGKK), which can be cleaved by PC2 in the residues of human proGIP²². Therefore, ProGIP could be processed not only to GIP1-42 in the gut, but also to the shorter fragment GIP by PC2, then ultimately to GIP1-30 amidated by the action of peptidyl-glycine α -amidating monooxygenase in pancreatic α -cells.

In the perfusion study of mouse pancreas, GIP1-30 showed almost equal capacity of concentration-dependent insulin secretion compared with GIP1-42²². GIP1-30 also promoted somatostatin release from the perfused stomach at pharmacological concentrations similar to GIP1-42²³. Thus, GIP1-30 presented equivalent physiological actions to GIP1-42. Active GIP was detectable from the culturing media of isolated islets from mice and humans by a GIP receptor-mediated cAMP 3',5'-cyclic adenosine monophosphate assay with a CRE-luciferase construct. In addition, GIP neutralizing antibody or GIP receptor antibody diminished glucose-stimulated insulin release from the isolated islet, suggesting that GIP induced insulin release in an "intra-islet" paracrine manner²².

Unexpectedly, co-immunoreactivity of GIP and PC2 was also observed in the gut when the antibody against the midportion of GIP, but not the C-terminal GIP antibody¹⁵ (3.65H), was used for detection, suggesting that a subset of gut endocrine

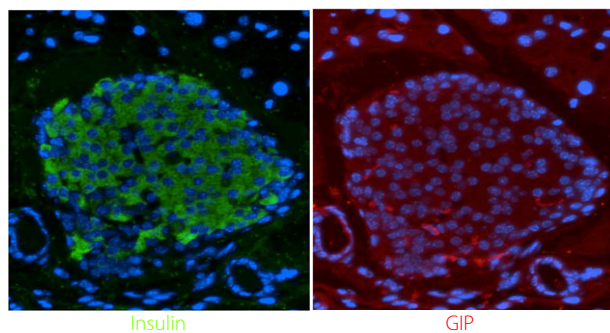


Figure 3 | Glucose-dependent insulinotropic polypeptide (GIP)1-42 is not expressed in the pancreatic islet. Immunohistochemistry of a wild-type mouse islet. Insulin (green), GIP (red) and nuclei (blue). Note no GIP1-42 immunoreactivity in the islet. Monoclonal C-terminal specific antibody against GIP1-42 (3.65H) was used for detection¹⁵ (magnification: $\times 400$).

cells are secreting GIP1-30, but not GIP1-42, through ProGIP processing by PC2²³.

In the developing pancreas of the human fetus, GIP expression was also found in pancreatic β -cells²². Intense GIP immunostaining was sparse, but largely coincident with insulin immunoreactivity at 10 weeks of fetal age. GIP immunoreactivity was more prevalent and largely restricted to α -cell areas at 15 weeks. Another study showed that GIP mRNA and immunoreactivity were detected in the islets of mouse embryos by reverse transcription polymerase chain reaction or by immunohistochemistry. Both antisense and small interfering RNA against GIP decreased the number of Pdx-1-positive and sox9-positive cells, and inhibited the differentiation of β -cells in the cultured embryonic pancreas, suggesting that pancreatic GIP might play a key role in the development of pancreatic islets²⁴. Furthermore, we preliminarily presented in the symposium “*Incretin 2015*” that GIP1-30 expression was enhanced with α -cell expansion concomitantly with decreased β -cell area in diabetic rodent models including NOD (Non Obese Diabetes), Akita and low-dose streptozotocin (LD-STZ) mice (YF, TY, YT, JH, YT, AA, YM, HM). Further studies are necessary to elucidate the mechanism of enhanced GIP1-30 expression in diabetes.

THERAPEUTIC POTENTIAL OF GIP1-30

It is crucial to investigate whether GIP1-30 has the therapeutic potential to treat diabetes and to protect β -cells against chronic hyperglycemia. It is also interesting whether GIP1-30 has different actions to extrapancreatic tissues compared with GIP1-42. Widenmaier *et al.*²⁵ synthesized a dipeptidyl-peptidase 4-resistant GIP1-30 (D-GIP1-30), and evaluated its effects on glucose homeostasis and preservation of β -cell mass in several rodent diabetic models. D-GIP1-30 improved acute glucose tolerance and insulin secretion in both lean and obese rats during the intraperitoneal glucose tolerance test. In addition, chronic treat-

ment with D-GIP1-30 inhibited β -cell apoptosis, preserved β -cell mass, and improved postprandial glycemia and insulin secretion in STZ rats and Zucker diabetic fatty rats. Interestingly, exogenous D-GIP1-30 did not induce bodyweight gain. In *in vitro* studies, D-GIP1-30 greatly reduced action on lipoprotein lipase activity in cultured 3T3-L1 adipocytes compared with GIP1-42, although GIP1-30 equivalently promoted insulin secretion and inhibited β -cell death against staurosporine.

We preliminarily presented in the symposium “*Incretin 2015*” that the long-acting GIP1-30 with PEGylation could suppress the progression to hyperglycemia and improve glucose tolerance in LD-STZ mice. The GIP1-30 analog did not affect glycemia in non-diabetic mice, but reduced glycated hemoglobin levels and improved glucose excursions after an intraperitoneal glucose tolerance test in LD-STZ mice, compared with non-treated LD-STZ mice. The GIP1-30 analog also reduced α -cell expansion in the islets and suppressed plasma glucagon levels compared with non-treated LD-STZ mice (unpubl. data). Taken together, we might be able to expect therapeutic potentials of GIP1-30 for some diabetic conditions.

CONCLUSION

Collectively, GIP1-30 might play important roles in glucose metabolism including insulin secretion and islet protection. However, further studies should be carried out to elucidate the mechanism of secretion and actions, pathophysiology, and the therapeutic potentials of GIP1-30.

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DISCLOSURE

The authors declare no conflict of interest.

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