Secretion of Gut GLI and Glicentin

Akira OHNEDA

Health Center, Tohoku University, Sendai 980, Japan

Received March 15, 1995; final version accepted May 30, 1995

Intraluminal administration of nutrients to piglets elicited an enhancement of glicentin secretion. These results correspond with the findings obtained in experiments with dogs in terms of gut glucagon-like immunoreactivity (GLI). Although the response of pancreatic glucagon to hypoglycemia is well known, the experiment with piglets demonstrated the secretion of gut glicentin in response to hypoglycemia. Other experiments with dogs indicated that GLI secretion in response to hypoglycemia or neuroglycopenia is regulated by the parasympathetic system. However, in the postprandial state, the effect of the nervous system is weak and is overcome by the secretion of glicentin in response to the intraluminal nutrients. Ileojuninal transposition revealed intestinal adaptation, where an elevated plasma glicentin or GLI, thickening of the intestinal mucosa and an increased content of glicentin in the intestine were observed. The response of gut GLI to glucose was also exaggerated in the ileojunial transposition dogs. These results suggest that the animal model with intestinal adaptation such as ileojunial transposition will be useful for further research on glicentin secretion.

KEYWORDS: glicentin, glucagon, glucagon-like immunoreactivity, nutrients, nervous system, hypoglycemia, intestinal adaptation

1. Introduction

It has been well known that glicentin is processed in the gut from preproglucagon, a common precursor of pancreatic glucagon, in addition to glucagon-like peptides 1 and 2. Since glicentin is a major peptide responsible for gut glucagon-like immunoreactivity (GLI) secreted from the gastrointestinal tract, the regulation of glicentin secretion has interested many researchers in this field.

The source of glucagon was long regarded as the islet of the pancreas until 1948, when Sutherland and de-Duve demonstrated the presence of glucagon (hyperglycemic glycogenolytic factor) in the gastrointestinal tract, using a bioassay method. This was confirmed using a radioimmunoassay for glucagon by Unger et al. (1966), who described glucagon in the gastrointestinal tract and then coined the term 'glucagon-like immunoreactivity' (GLI) (Unger et al. 1968). Further studies by gel chromatography demonstrated that gut GLI is heterogeneous and is divided into at least two components peak 1, with larger molecular weight; and peak II with smaller molecular weight (Valverde et al. 1968). In 1976, the amino acid structure of peak 1 was partially analysed and this material was called glicentin, because of its composition from one hundred amino acids residues (Sundby et al. 1976). Although the molecular structure of peak 1 was determined to be only 69 amino acid peptide in 1981, the name glicentin remains (Thim, Moody 1981).

Developments through molecular biology have demonstrated that both pancreatic glucagon and gut glicentin are produced from a common precursor, preproglucagon, and that various other peptides are produced through different processing in the islet of the pancreas and in the gut, as shown in Fig. 1 (Lund et al. 1982; Bell et al. 1983): glicentin related pancreatic peptide (GRPP) in the pancreas; and, in the gut, in addition to glicentin, oxyntomodulin, GRPP, and glucagon-like peptides (GLP) 1 and 2.

Although the biological action and secretion of pancreatic glucagon are widely known, secretion of glicentin or gut GLI has not been fully clarified. The present paper will summarize our current understanding, using the results from various experiments of the organization and regulation of glicentin secretion in different physiological states.

2. Measurements of glucagon-related peptides

The biological action of glucagon was clarified to increase blood glucose through glycogenolysis, stimulating adenylcyclase, and resulting in an increase of cAMP in the liver, or to stimulate lypolysis. Measurements of glucagon were therefore performed by bio-aasays based on these biological actions. However, almost all bioassays, in vivo or in vitro, were insufficient to measure circulating glucagon in terms of specificity, sensitivity or reproducibility. Soon after Berson et al. developed the radioimmunoassay for insulin in 1956, Unger and his co-workers perfected a radioimmunoassay method for measuring glucagon, overcoming many difficulties to successfully produce anti-glucacon antisera in animals (Unger et al. 1959). Thereafter, this radioimmunoassay
method was repeated in several laboratories around the world (Grodsky et al. 1959, Samols et al. 1965, Lawrence 1966, Heding 1969). As a result, knowledge concerning the states of circulating glucagon accumulated rapidly and a much clearer understanding was reached.

In 1966, Unger and his co-workers measured the contents of glucagon in the pancreas and the gastrointestinal tract in dogs, showing that nearly same amount of glucagon was contained in the intestine, in total, as in the pancreas. This indicated that the radioimmunoassay method measured both pancreatic and gut glucagon, but Unger et al. separated pancreatic glucagon from gut glucagon-like immunoreactivity, although at that time these two immunoreactivities could not be differentiated. In 1968, Eisenbraut and her co-workers reported that several antisera produced against glucagon in their laboratory could discriminate glucagon-like immunoreactivity in the gut from pancreatic glucagon. The antisera which could distinguish pancreatic glucagon were produced in rabbits only by chance, but several laboratories obtained such antisera, as 30 K, K47 and G21. Thereafter, glucagon immunoreactivity measured with such discriminating antisera was called pancreatic glucagon, while that measured with non-specific antisera was coined gut glucagon-like immunoreactivity (GLI), total immunoreactive glucagon (IRG) or enteroglucagon.

In 1972, Assan and Slusher studied the structure/immunoreactivity relationships of the glucagon molecule and concluded that non-specific antisera react with the N-terminal and central portion of glucagon, whereas specific antisera bind with the C-terminal portion.

In our laboratory, we tried to produce such antisera specific to pancreatic glucagon using the C-terminal fragment as an antigen and succeeded in producing a specific antiserum (Ohenda et al. 1979). Thereafter, specific antisera for pancreatic glucagon could be easily produced in animals using the C-terminal fragments of glucagon (Nishino et al. 1981). Furthermore, it became clear that specific antisera do not bind with the C-terminal portion of the glucagon molecule of gut GLI, because it is masked with the peptides connected to this terminal portion (Moody et al. 1978).

After the molecular structure of glicentin was confirmed (Thim, Moody 1981), and common processing of preproglucagon in the pancreas and L-cells in the small intestine was discovered (Lund et al. 1982, Bell et al. 1983), the relationship between the molecular structure and antisera used in the measurements of glucagon-related peptides has clearly been understood, as shown in Fig. 2. As presented here, the substance formerly called gut GLI includes glicentin, oxymotodulin and GRPP plus the N-terminal portion of glucagon. Furthermore, glicentin was demonstrated as the main source of GLI in the L-cells in the intestine. Therefore, we are keenly interested in glicentin among the gut GLI-related materials. At present we have three types of antisera, available in the measurement of glicentin, R64, non-specific antisera and antisera to C-terminal glicentin (residues 62–69). Among these, R64 antiserum reacts with the C-terminal portion of GRPP, which shows high species specificity (Thim, Moody 1981, Lund et al. 1982, Bell et al. 1983). That is, R64 antiserum was produced against porcine
<table>
<thead>
<tr>
<th>Preproglucagon</th>
<th>GRPP</th>
<th>Glucagon</th>
<th>GLP-1</th>
<th>SP-2</th>
<th>GLP-2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Posttranslational Processing</strong></td>
<td>1</td>
<td>30 33</td>
<td>61 72</td>
<td>158</td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>GRPP</td>
<td>Glucagon</td>
<td>MPGF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antisera</td>
<td>R64</td>
<td>NS</td>
<td>SA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small Intestine</td>
<td>Glicentin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 30 33 69</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antisera</td>
<td>R64</td>
<td>NS</td>
<td>CA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS: Cross reacting glucagon antiserum such as G25
SA: Pancreatic glucagon specific antiserum such as G21, 30K, K47
CA: Specific antiserum to the C-terminal portion of glicentin

Fig. 2 Relationships between molecules of glucagon-related peptides and the antisera for their measurement.

Glicentin and is useful only to measure porcine glicentin.

### 3. Secretion of Gut GLI and glicentin in response to nutrients

It is well known that in the L cells of the intestine glicentin is processed from the precursor of glucagon-related peptides. Since the L cells are localized in the epithelium of the microvilli, glicentin is presumed to be released in response to the intraluminal nutrients. The discrepancy of circulating glucagon-like immunoreactive material (GLI) in response to intravenous and intraduodenal administration of glucose (Unger, Eisenraut 1967) prompted many researchers to investigate the characterization of GLI in the blood.

Earlier work reported that plasma glucagon was elevated following the oral administration of glucose in man (Samols et al. 1965). Soon after, the increase in plasma glucagon (GLI) was proven to derive from the intestine in response to the intraluminal load of glucose (Unger et al. 1968). When glucose was administered orally to normal dogs in a conscious state, blood glucose rose and total IRG increased, as shown in Fig. 3 (Ohneda et al. 1983). In this experiment, plasma insulin increased whereas plasma IRG did not change after the glucose load. These results indicated that intraluminal administration of glucose enhanced the release of gut GLI, whereas the secretion of pancreatic glucagon either was not affected or was rather inhibited.

In order to investigate the response of gut GLI to glucose load much more directly, the response of glicentin was studied in piglets using R64 antiserum, which reacts with the N-terminal portion of glicentin (Moody et al. 1981). The response to the intraduodenal administration of glucose in piglets is presented in Figs. 4A and B (Ohneda 1987). Plasma insulin rose gradually and plasma IRG rose slightly, after the glucose load. In contrast, plasma total IRG and glicentin increased markedly and continued to rise during the 90 min following glucose ad-
ministration. These results clearly indicated that intraluminal glucose stimulates the secretion of glicentin from the L cells of the intestine. Since alpha anomer of glucose stimulates the secretion of GLI from the gut much more than the beta anomer (Matsuyama et al. 1979), it is suggested that the glucoreceptor in the L cell plays an important role in the release of glicentin.

Concerning the effect of fat ingestion on plasma GLI, no reports were published until 1971, when it was observed that oral loading of butter caused a rise in total IRG in human plasma (Sato et al. 1971). In 1973, Böttiger et al. reported an increase in the levels of both pancreatic glucagon and total IRG following the intraduodenal administration of peanut oil in dogs. Soon after, the increased release of gut GLI following intraluminal instillation of butter was confirmed in our laboratory (Ohneda et al. 1975). In both studies, pancreatic glucagon, in addition to gut GLI, was elevated following triglyceride loading, this finding being interpreted as the secondary effect of gut factors stimulated after fat ingestion.

It was reported that the intraluminal administration of triglyceride did not stimulate the release of gut GLI in pancreatectomized dogs (Ohneda et al. 1984). Further study showed that hydrolysed products of triglyceride, glycerol and free fatty acids, enhanced the release of gut GLI (Ohneda et al. 1984). Among free fatty acids, long chain fatty acid stimulates GLI release whereas medium chain fatty acid does not (Ohneda et al. 1984).

In order to elucidate the secretion of glicentin in response to triglyceride ingestion, experimental studies were carried out in piglets using antiserum R64, reacting with the N-terminal portion of glicentin (Ohneda et al. 1987b). The changes in response to butter load are presented in Figs. 5A and B. Plasma triglyceride and insulin increased only slightly after butter load, but plasma total IRG and glicentin were markedly elevated.

In 1968, we observed an elevation of plasma glucagon following the intraduodenal administration of an amino acid mixture in dogs (Ohneda et al. 1968). From comparison of the changes in plasma glucagon in the vena cava, pancreaticoduodenal vein and mesenteric vein, it was concluded that the increased plasma glucagon fol-
Secretion of Gut GLI and Glicentin

Figs. 4A and B  Changes in blood glucose and plasma IRI (A) and plasma IRG, total IRG and glicentin (B) during intraduodenal administration of glucose in a group of six normal conscious pigs.

Figs. 5A and B  Changes in blood glucose, plasma triglyceride, insulin (IRI) (A), IRG, total IRG and glicentin (B) during intraduodenal administration of butter in a group of six piglets.
lowing amino acids derived from the pancreas but not from the intestine. However, the experimental study in piglets showed that the amino acid mixture administered into the duodenum enhanced the release of glicentin from the intestine, as shown in Figs. 6A and B (Ohneda et al. 1988). Furthermore, oral administration of arginine induced a marked increase in total IRG but little changes in plasma glucagon in pancreatectomized dogs (Ohneda et al. 1988). The discrepancy in glicentin response to amino acids between the former observation in dogs and the recent experiment using piglets and dogs can be interpreted by the use of different antisera. In the previous study, we used an antiserum to glucagon (G128P), which cross-reacts with intestinal GLI less than 25%, while in the recent study antisera used were specific to the C-terminal of glucagon (G21) and crossreactive with gut GLI (G25). G128P antiserum might not have measured GLI from the intestine, as G21 could not.

From these studies, it is concluded that nutrients, carbohydrate, triglyceride and amino acids administered into the intestine enhance the release of glicentin from the gut. The comparison of glicentin responses indicates that glucose provides the strongest response, followed by triglyceride and amino acids.

In addition, the intraluminal administration of calcium, magnesium and deionized water are known to enhance the release of gut GLI (Böttger et al. 1972, Matsuyama et al. 1981).

4. Response of gut GLI to hypoglycemia

Experiments in the 1960's demonstrated clearly that hypoglycemia stimulates the release of glucagon from the pancreas, resulting in increased glucose output from the liver (Unger et al. 1962, Ohneda et al. 1969). However, at that time, little was known about the response of gut GLI to hypoglycemia.

In order to investigate the response of gut GLI, antiserum R64 was used in the experiment for a much more specific measurement of glicentin. Because the C-terminal portion has a different amino acid structure in different animal species and R64 antiserum was produced against porcine glicentin, piglets were used in the study to see the response to hypoglycemia. As shown in Fig. 7, insulin-induced hypoglycemia elicited clearly an increase in plasma glicentin, measured with R64 antiserum (Ohneda et al. 1987a). Since it was reported that GRPP was elevated in the pancreatic vein during arginine infusion in a perfusion experiment with porcine pancreas (Moody et al. 1981), the increase in plasma glicentin measured with R64 in response to hypoglycemia might reflect the release of GRPP and glucagon from the pancreas. Gel chromatography of porcine plasma obtained during
hypoglycemia revealed that increased plasma glicentin was mainly eluted at the glicentin marker (Ohneda et al. 1987a).

The response of gut GLI to hypoglycemia was further investigated in pancreatectomized dogs in a fully conscious state (Ohneda et al. 1989). As shown in Figs. 8A and B, plasma total IRG increased markedly during insulin-induced hypoglycemia. In addition, chromatography of plasma obtained during hypoglycemia showed the main peak of total IRG at the glicentin marker. Therefore, it could be concluded from these studies that hypoglycemia stimulates the release of glicentin or GLI from the gut in pigs and dogs. This was the first demonstration that hypoglycemia stimulates the release of glicentin.

Characterization of plasma glicentin in response to hypoglycemia was performed in dogs for convenience, using both the antisera specific to the C-terminal and N-terminal portion of glucagon (Ohneda et al. 1989). Under anesthesia, insulin-induced hypoglycemia did not elicit significant increase in plasma IRG or total IRG in pancreatectomized dogs. Furthermore, the plasma levels of IRG or total IRG did not change significantly during insulin-induced hypoglycemia in normal dogs under anesthesia. These results suggest that the nervous system plays an important role in the response of pancreatic glucagon and gut GLI to hypoglycemia.

In order to investigate the effect of neurotransmitter on GLI secretion from the gut, epinephrine was infused into dogs, resulting in a slight increase in total IRG. In contrast, acetylcholine clearly elevated the plasma levels of total IRG. To investigate the involvement of adrenergic agents in the response of gut GLI to hypoglycemia, adrenergic blockers were administered prior to the insulin injection. Neither phentolamine nor propranolol altered the response of plasma total IRG to insulin-induced hypoglycemia. In contrast, the administration of atropine completely blunted the response of gut GLI to insulin-induced hypoglycemia, as shown in Figs. 9A, B. These results indicate that the cholinergic system plays an important role in the response of gut GLI to hypoglycemia in dogs. Furthermore, enhanced secretion of gut GLI by 2-deoxy-D-glucose-induced neu-
Figs. 8A and B  Changes in blood glucose (BG), plasma IRG and total IRG during hypoglycemia in a pancreatectomized dog. Insulin injection (44 U) resulted in a decrease of BG to 51 mg/100 ml at 135 min (A). Gel chromatography of plasma obtained during insulin hypoglycemia in a pancreatectomized dog. The column was calibrated by blue dextran (BD), and labeled glicentin (Gli), insulin (I), and glucagon (G) (B).

Fig. 9  A. Changes in blood glucose (BG), plasma IRG, and total IRG during insulin-induced hypoglycemia in a group of 10 normal dogs. B. 0.5 mg of atropine was intramuscularly administered prior to insulin injection in the same group of 10 dogs.
roglycopenia was also completely abolished by the administration of atropine. These results with 2-deoxy-D-glucose support the idea that the secretion of gut GLI is enhanced through the parasympathetic system in response to hypoglycemia.

The biological action of GLI released during insulin-induced hypoglycemia is unclear at present. Glicentin does not displace labeled glucagon to the liver cells (Moody et al. 1980). The administration of synthetic human glicentin does not increase blood glucose in dogs (Ohneda et al. 1995). Therefore, biological actions of glicentin other than hyperglycemia should be investigated in the future.

5. A role of vagus nerve in GLI secretion

Hitherto, the secretion of gut GLI has been studied concerning the response to intraluminal administration of nutrients (Ohneda 1974). However, intravenous administration of bombesin elicited an increase in plasma GLI (Kaneto et al. 1978). Furthermore, as mentioned in the previous chapter, GLI secretion in response to hypoglycemia or neuroglycopenia is evoked through the parasympathetic nervous system. These findings suggest a role for the vagus nerve in the secretion of gut GLI.

To investigate this further, firstly the effect of atropine upon the glucose-induced response of gut GLI was investigated in conscious dogs (Ohneda et al. 1985). To avoid the influence of gastric juice, two Thomas cannulae were attached at the anterior wall of the stomach and at the opposite side of the duodenal papilla a week prior to the experiment. After an overnight fast, both the ventricular and duodenal fistulae were opened and a 20% solution of glucose (2 g/kg), with or without atropine, was administered into the duodenum through a catheter. The changes in blood glucose, plasma insulin, glucagon and total IRG(GLI) are presented in Fig. 10. The response of total IRG was slightly reduced, though not significantly, in the atropine group.

Secondly, a glucose tolerance test was carried out in vagotomized dogs, in which the dorsal and ventral trunks of the vagus nerve were cut below the diaphragm and pyloroplasty was carried out a week prior to the experiment. There was no difference in blood glucose, plasma insulin, IRG and total IRG compared with those in the normal dogs, except for a slight reduction of plasma total IRG.

Finally, the direct effect of the vagus nerve on the secretion of gut GLI was investigated. After an overnight

![Graph showing changes in blood glucose (BG), plasma IRI, IRG and total IRG in the jugular vein following intraduodenal administration of glucose combined with atropine in a group of 5 normal dogs.](image)
fast, both the dorsal and ventral trunks of the vagus nerve were cut below the diaphragm. After approximately 60 min rest, an electrode was attached to the peripheral site of the dorsal trunk of the vagus nerve and an electrical stimulus was given for 10 min. Forty min after the start of the first stimulus, 1 mg of atropine was given intravenously and 10 min later the second electrical stimulus was given. A typical result is presented in Fig. 11. The electrical stimulus increased plasma insulin, IRG and total IRG slightly. However, the electrical stimulus following atropine injection did not induce any rise of plasma insulin, IRG or total IRG.

There was no significant difference in the fasting level of plasma total IRG between the normal and atropine or vagotomized groups. However, the peak level of plasma total IRG after glucose instillation seems to be lower in the atropine or vagotomized group. The reduction of gut GLI secretion in a state of vagal blocking might be interpreted by a decrease in responsiveness of the L cells.

The electrical stimulus caused a slight increase of insulin and glucagon secretion from the pancreas, as well as GLI release. Furthermore, atropine administration blunted these responses. These findings suggest the innervation of the L cells by the vagus nerve. These results indicate that the vagus nerve influences the secretion of GLI and its blockade induces the reduction of GLI response. However, the effect of vagus nerve is not strong, so that it might be masked by the effect of nutrients ingested into the gut.

6. Secretion of gut GLI during intestinal adaptation

Experimental and clinical studies have demonstrated that following partial resection of the small bowel the residual intestine shows adaptive changes, which include mucosal hypertrophy, increased absorptive function and a deviation of circulating gastrointestinal hormones (Dowling, Booth 1967, Bloom, Polak 1982). Therefore, the changes in plasma GLI and metabolic derangements were investigated during the course of intestinal adaptation, which was induced by ileojejunal transposition (IJT) in dogs, as shown in Fig. 12 (Ohneda et al. 1988a, 1990).

Although fasting levels of blood glucose, plasma insulin, gastric inhibitory polypeptide, and IRG did not change significantly throughout the 12 weeks, plasma total IRG increased gradually and reached a peak of 634 pmol/l at the 8th week. Responses to oral glucose load at the 12th week are presented in Fig. 13. The plasma level of total IRG was markedly elevated after glucose load and reached higher than 3000 pmol/l.

The IJT animals revealed an exaggerated response of total IRG to insulin-induced hypoglycemia. At the 12th week, plasma total amino acids were significantly reduced in IJT animals and a marked reduction was observed in serine, proline, glycine, alanine, tyrosine and arginine.

The small bowel in the group of IJT was markedly thickened in comparison with the sham group, especially in the jejunum and the transposed ileum. The mean thickness of the mucosal layer in the IJT group increased in
Fig. 12 Experimental models. In ileojejunal transposition, a distal quarter of the small intestine (B'-C, I) was isoperistaltically transposed to the jejunum (A-A') 20 cm distal to the ligament of Treitz (TL).

Fig. 13 Changes in blood glucose (BG), plasma IR1, GIP, IRG, and total IRG during oral glucose load 12 weeks after the operation in the IJT group. The shadows or dot lines show the changes in a group of 4 dogs with sham operation.

all parts of the gut, especially in the transposed ileum, in comparison with that of the sham group.

Glucagon content in each divided part of the gut are presented in Fig. 14. IRG content measured with the specific antiserum increased slightly in all parts of the IJT group, in comparison with that in the sham group. Total IRG measured with the non-specific antiserum increased markedly in all parts in the IJT group, compared with that in the sham group.

The IJT animal model revealed an exaggerated response of plasma total IRG to oral glucose load and insulin-
induced hypoglycemia, suggesting that the release of gut GLI is enormously enhanced during intestinal adaptation.

Chromatography of total IRG of plasma and extracts from the small intestine of IJT group at the 12th week showed a main peak at the glicentin marker: i.e., this suggests that plasma total IRG released from the gut in response to oral glucose load consists mainly of glicentin. Furthermore, mucosal hyperplasia and an increase in total IRG (glicentin) in the small intestine were proven in the IJT group. These findings suggest that increased plasma total IRG (glicentin) derived from the gut.

At present, the physiological roles of glicentin are not known. There has been only one reported case of enteroglucagonoma, which suggests the biological action of glicentin, disturbance of bowel movement and hypertrophy of the intestinal mucosa (Glæson et al. 1971). The findings in the present experiment indicate clearly that the elevated plasma level of GLI (glicentin) relates to the reduction of plasma amino acids and the morphological changes in the intestine.

7. Several problems in the field of glicentin research

The measurement of glicentin has not prevailed in general. Hitherto, gut glucagon-like immunoactivity (GLI) has been measured by radioimmunoassay using non-specific or N-terminal glucagon sensitive antisera. Therefore, GLI measured by such antisera includes pancreatic glucagon, N-terminal peptides of glucagon, GRPP plus N-terminal glucagon and oxyntomodulin in addition to glicentin. The measurement of glicentin for many animal species remains to be developed in the future.

In the study using pigs, plasma glicentin was determined with the antiserum R64, which reacts with the C-terminal portion of GRPP. Therefore, the value of plasma glicentin obtained would be considered much more specific to glicentin compared with those determined by non-specific antisera for glucagon. However, according to the present study, the changes in glicentin response correspond well with the results reported previously in terms of gut GLI or total IRG (Unger et al. 1968; Ohneda et al. 1975; Ohneda 1988). Therefore, we could figure out the changes in plasma glicentin in many circumstances from the results expressed with gut GLI or total IRG.

The secretion of gut GLI or glicentin has been studied for a long time by various researchers. As assumed from the anatomical distribution of L cells, intraluminal nutrients stimulate strongly the release of gut GLI. In addition, several factors are involved in the release of glicentin, as demonstrated in hypoglycemia. However, the development of a new assay method for glicentin might expand our knowledge concerning the secretion of glicentin. Intestinal adaptation models such as IJT will continue to be useful tools. As a result of such experi-
ments, the physiological action of glicentin will be elucidated in the future.

Acknowledgments

The author is grateful to Drs. Takashi Kobayashi, Jiro Nihei, Makoto Ohneda, Iwao Sasaki, Takashi Tsuchiya and Hiroo Naito, Tohoku University School of Medicine for their skillful assistance. The study was supported in part by a Scientific Grant from the Ministry of Education, Science and Culture, Japan (Nos. 437048, 57570848, 58570985 and 857092).

REFERENCES