Insulinotropic Action of Human Glicentin in Dogs

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Glicentin has been demonstrated to be released in response to the intraluminal administration of nutrients, but its biological action remains unknown. To clarify the effect of glicentin on the endocrine function of the pancreas, the present study was performed using an in vivo local circulation system of the canine pancreas. During infusion of 0.5% solution of glucose or arginine, 100 and 400 pmol glicentin and 400 pmol glucagon were administered into the pancreaticoduodenal artery (PA) within 10 minutes at 40-minute intervals successively. During glucose infusion, blood glucose in the femoral artery did not change following administration of 100 pmol glicentin, but slightly increased following 400 pmol glicentin. Plasma insulin (immunoreactive insulin [IRI]) in the pancreaticoduodenal vein (PV) increased significantly only following infusion of 400 pmol glicentin. Plasma glucagon (immunoreactive glucagon [IRG]), measured with a specific antiserum to the C-terminal portion of glucagon, did not change following administration of 100 pmol glicentin, but was slightly elevated following 400 pmol glicentin. Plasma total IRG, measured with a nonspecific antiserum, increased promptly after administration of 100 and 400 pmol glicentin. During arginine infusion, the response of plasma IRI to glicentin was markedly exaggerated both in dosages of 100 and 400 pmol. From the present study it was concluded that human glicentin clearly increases insulin release from the canine pancreas.

CHARACTERIZATION of glucagon-like immunoreactivity (GLI) isolated from porcine intestine demonstrated the primary structure of a peptide, glicentin, with 69 amino acid residues and a molecular weight (MW) of 8,128.[1] In 1983, Bell et al[2] determined the structure of hamster pancreatic preproglucagon with 180 amino acid residues (MW 18,000) from the sequence of its cDNA, suggesting that glucagon-containing polypeptides in the pancreas and nonpancreatic organs may be derived from a common precursor. Indeed, the preproglucagon gene has been shown to be expressed both in the pancreas and the intestine, and it was proved that mRNAs for preproglucagon are identical in the pancreas and intestine.[3] Research on glucagon-related peptides revealed that preproglucagon is processed in the L cell of the intestine, yielding glucagon-like peptides 1 and 2 in addition to glicentin.[4,5] Therefore, it is presumed that glicentin is an important peptide among gut glucagon-related peptides. According to our studies, the circulating level of plasma glicentin reaches approximately 1 nmol/L following nutrient ingestion.[6] Therefore, the plasma concentration of glicentin is far higher in comparison to those of the other gastrointestinal hormones, suggesting some biological actions. However, the physiological action of glicentin has not been clarified yet. Since recombinant human glicentin has recently become available,[7] we have studied its insulin-releasing action in dogs.

MATERIALS AND METHODS

The effects of human glicentin on the endocrine function of the pancreas were investigated using an in vivo local circulation system of the canine pancreas, as reported previously.[8]

The human glicentin used in the present study was produced by a recombinant DNA method as reported previously.[7] Briefly, the gene coding for human glicentin, a 69-residue polypeptide, has been designed and chemically synthesized based on the amino acid sequence deduced by Bell et al[9] from the genomic sequence of human preproglucagon. This gene was ligated to an expression vector pKK233-2 and expressed in *Escherichia coli* strain SG21059. Human glicentin produced in *E coli* was extracted by sonication and finally purified to produce a single peak in sodium dodecyl sulfate–polyacrylamide gel electrophoresis and reverse-phase high-performance liquid chromatography analysis. The entire amino acid sequence of purified human glicentin was determined with an automated protein sequencer (Shimadzu model PSQ-1, Kyoto, Japan) after digestion with lysylendopeptidase. The result was exactly as expected, based on the amino acid sequence of human glicentin from the genomic DNA, except for an additional methionine residue at its *N*-terminal.[9]

In the present study, 12 healthy mongrel dogs weighing 11 to 13 kg were studied. After an overnight fast, dogs were anesthetized with pentobarbital sodium and the abdomen was opened by a midline incision. A glass T-cannula connected with a Teflon catheter was inserted into the superior pancreaticoduodenal artery (PA). Plastic needles were inserted into the superior pancreaticoduodenal vein (PV) and the femoral artery. Approximately 1 hour after the operation, the experiments were started. After the initial samples were drawn, 0.5% glucose solution or 0.5% arginine solution (Morishita Pharmaceutical, Tokyo, Japan) was infused into the PA at a rate of 2 mL/min. Twenty minutes after the start of glucose or arginine infusion, recombinant human glicentin (calculated MW 8,232) in a dose of 100 or 400 pmol and porcine glucagon (MW 3,485, Eli Lilly & Co, Indianapolis, IN) in a dose of 400 pmol were successively administered into the PA within 10 minutes at 40-minute intervals. To avoid adsorption of the peptides to the tubing, bovine serum albumin (Rehis Chemical, Phoenix, AZ) was added to each solution at a concentration of 0.2%. Blood samples were obtained before and 1, 3, 6, 10, 15, 20, 30, and 40 minutes after the start of the peptide infusion. For hormone assay, 4 mL blood was drawn from the PV and collected into a glass tube containing 1,000 KIU aprotinin (Trasylol; Bayer, Leverkusen, Germany) and 10 mg EDTA in ice. After completion of the experiment, plasma was separated by centrifugation at 4°C and stored at −20°C until the assay began. For glucose determination blood was obtained from the femoral artery.

Plasma insulin (immunoreactive insulin [IRI]) was determined by radioimmunoassay with the two-antibody system.[10] The minimum detectable dose for IRI was 0.2 μU per tube.[11] The plasma glucagon (immunoreactive glucagon [IRG]) level was measured by radioimmunoassay using antiserum G-21 specific to the C-terminal

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portion of glucagon, as reported elsewhere.[12] Furthermore, plasma total IRG was determined with antiserum G-25 cross-reacting with pancreatic glucagon and gut GLI.[13] The detection limits for IRG and total IRG were 3 and 5 pg per tube, respectively.[11] For the hormone assays, porcine insulin and porcine glucagon donated by Eli Lilly & Co were used for the standard materials. Blood glucose was determined with the glucose oxidase method.[14]

For a comparison of the effects of glicentin and glucagon on the endocrine function of the pancreas, maximal changes in plasma IRI, IRG, and total IRG during 20 minutes after peptide infusion were calculated.

In the present study, the mean ± SEM values were calculated. Statistical analyses of changes in blood glucose, plasma IRI, IRG, and total IRG were performed with the Friedman test.[15] For a comparison of the maximal changes in plasma IRI, IRG, and total IRG, statistical analyses were performed with Student's *t* test.

RESULTS

Responses to Glicentin and Glucagon During Glucose Infusion

Changes in blood glucose, plasma IRI, IRG, and total IRG following administration of glicentin and glucagon in a group of six dogs are presented in Fig 1.

Blood glucose was 4.97 ± 0.24 mmol/L at baseline and did not change after infusion of glicentin at a dose of 100 pmol. However, 400 pmol glicentin elicited a slight increase in blood glucose from the initial level of 5.02 ± 0.29 to a peak of 5.57 ± 0.42 mmol/L at 3 minutes ($P < .05$). Blood glucose increased significantly from the baseline level of 5.04 ± 0.46 to 5.83 ± 0.62 mmol/L 15 minutes after administration of glucagon ($P < .01$).

Plasma IRI in the PV was 106 ± 12.4 mU/L before glicentin administration and slightly increased to 140 ± 30.3 mU/L 3 minutes after administration of 100 pmol glicentin, although the change was not significant due to wide deviations. In contrast, plasma IRI increased significantly from the baseline level of 96 ± 16.5 to 137 ± 14.2 mU/L 6 minutes after administration of 400 pmol glicentin ($P < .05$). Following administration of 400 pmol glucagon, plasma IRI increased from 116 ± 14.3 to a peak of 267 ± 34 mU/L at 6 minutes ($P < .001$).

Plasma IRG was 1,002 ± 259 ng/L at fasting and did not change significantly following infusion of 100 pmol glicentin. However, plasma IRG increased from the basal level of 918 ± 254 to 1,468 ± 349 ng/L 6 minutes after infusion of 400 pmol glicentin, returning to the initial level at 40 minutes ($P < .001$). Following administration of 400 pmol glucagon, plasma IRG increased from the baseline of 870 ± 204 to a peak of 3,592 ± 449 ng/L at 6 minutes ($P < .001$).

Plasma total IRG was 1,674 ± 395 ng/L initially and increased promptly to 2,965 ± 482 ng/L following infusion of 100 pmol glicentin ($P < .001$). Plasma total IRG increased to peaks of 3,800 ± 302 and 3,540 ± 501 ng/L after administration of 400 pmol glicentin and 400 pmol glucagon, respectively ($P < .001$).

Responses to Glicentin and Glucagon During Arginine Infusion

Changes in blood glucose, plasma IRI, IRG, and total IRG in a group of six dogs are depicted in Fig 2.

Blood glucose did not change following administration of glicentin at a dose of either 100 or 400 pmol, whereas it increased to a peak of 6.12 ± 0.53 mmol/L 20 minutes after administration of 400 pmol glucagon ($P < .05$).

Plasma IRI increased from the baseline level of 113 ± 27 to a peak of 355 ± 109 mU/L 3 minutes following administration of 100 pmol glicentin ($P < .05$). Administration of 400 pmol glicentin elicited an increase in plasma IRI from the preinfusion level of 120 ± 45.5 to a peak of 397 ± 113 mU/L ($P < .01$). After administration of 400 pmol glucagon, plasma IRI reached a peak of 397 ± 126 mU/L at 6 minutes ($P < .02$).

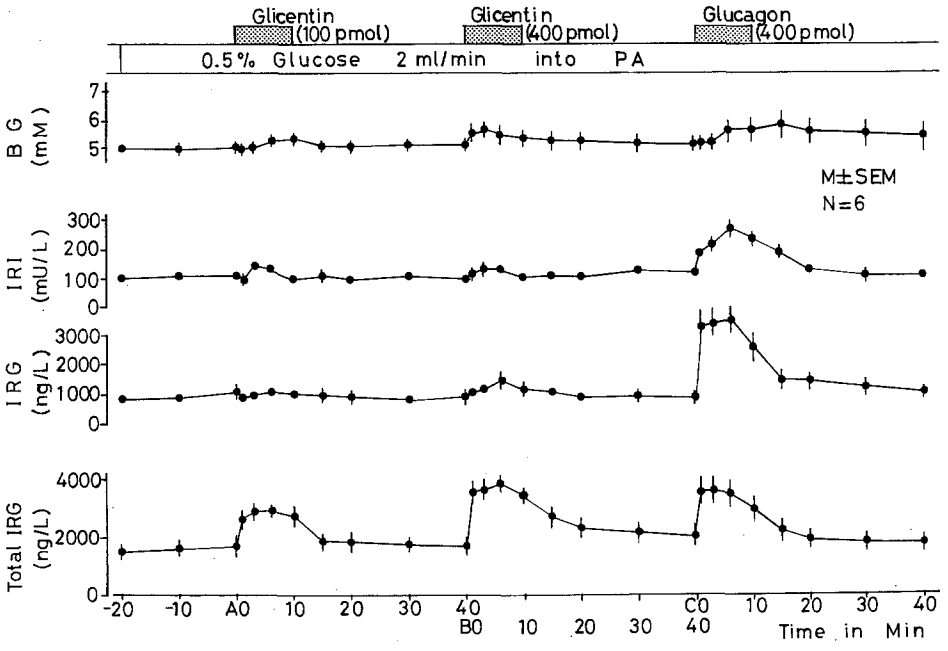

Fig 1. Changes in blood glucose (BG), plasma IRI, IRG, and total IRG following administration of 100 and 400 pmol human glicentin and 400 pmol glucagon during glucose infusion into the PA in a group of six dogs.

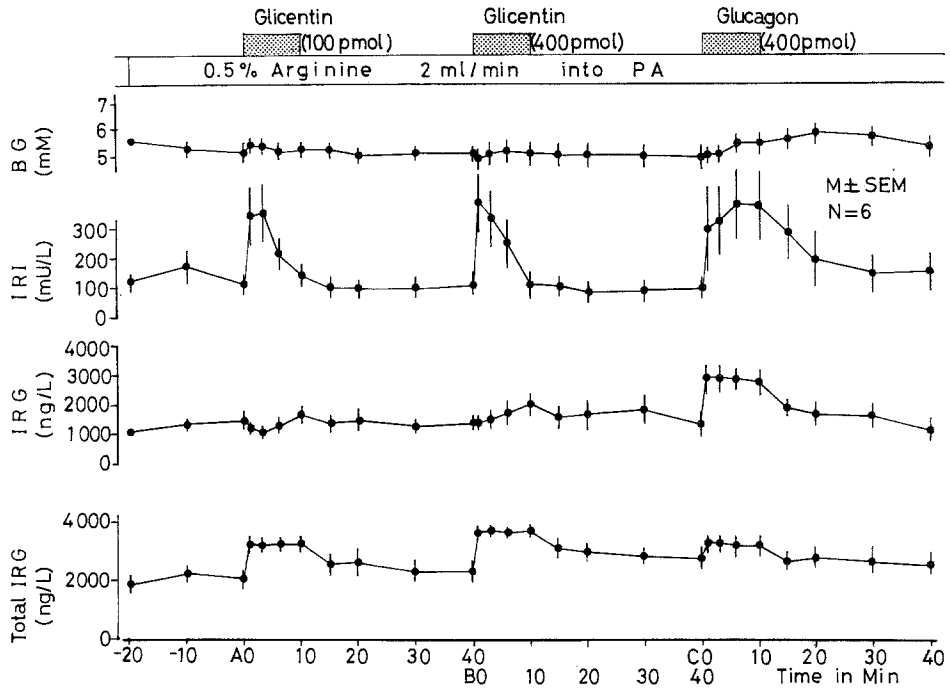

Fig 2. Changes in blood glucose (BG), plasma IRI, IRG, and total IRG following administration of 100 and 400 pmol human glicentin and 400 pmol glucagon during arginine infusion into the PA in a group of six dogs.

Plasma IRG increased from the fasting level of 1,082 ± 148 to 1,511 ± 367 ng/L 20 minutes after arginine infusion. Whereas plasma IRG did not change significantly following administration of 100 pmol glicentin, 400 pmol glicentin increased plasma IRG significantly to a peak of 2,035 ± 489 ng/L at 10 minutes ($P < .05$). Following the glucagon infusion, plasma IRG increased to a peak of 3,016 ± 446 ng/L at 1 minute ($P < .001$).

Plasma total IRG increased significantly following administration of 100 and 400 pmol glicentin and 400 pmol glucagon to peaks of 3,277 ± 284, 3,700 ± 192, and 3,385 ± 268 ng/L, respectively ($P < .001$).

Comparison of Responses to Glicentin and Glucagon

The maximal changes in plasma IRI, IRG, and total IRG following administration of these peptides during glucose infusion are presented in Table 1. The response of plasma IRI was not significant following administration of 100 pmol glicentin, whereas plasma IRI increased significantly after administration of 400 pmol glicentin or glucagon ($P < .01$). Plasma IRG increased slightly but significantly following administration of glicentin at a dose of 100 or 400 pmol, whereas glucagon infusion elicited a large increase of plasma IRG ($P < .01$). Plasma total IRG reached the high peaks following administration of each peptide ($P < .02$ or less).

During arginine infusion the response of plasma IRI was exaggerated, as shown in Table 2. Administration of glicentin at a dose of 100 or 400 pmol elicited similar changes in plasma IRI ($P < .05$). Plasma IRG did not change significantly following administration of glicentin at a dose of 100 or 400 pmol. However, glucagon infusion elicited a large increase of plasma IRG ($P < .05$). The maximal changes in plasma total IRG were observed following administration of glicentin in both dosages.

DISCUSSION

There have been several reports that deal with the insulin-releasing action of gut-derived glucagon-like immunoreactive materials. In 1968, Valverde et al[16] demonstrated the insulinotropic action of the second peak of gut GLI extracted from the canine intestine. Although they observed an insulin-releasing action of peak I of GLI with larger molecular weight, they could not exclude the possibility of contamination of gut peptides such as pancreozymin in peak I. In our laboratory, it was demonstrated that peak II of gut GLI with small molecular weight extracted from the canine intestine elicited an insulinotropic action, whereas

Table 1. Maximum Response to Glicentin and Glucagon During Glucose Infusion in Six Dogs

Peptide	Dose (pmol)	IRI (mU/L)		IRG (ng/L)		Total IRG (ng/L)	
		Basal	Maximal	Basal	Maximal	Basal	Maximal
Glicentin	100	106 ± 12.4	47.5 ± 21.8	1,002 ± 259	166.7 ± 34.5†	1,674 ± 394	1,454 ± 365*
Glicentin	400	95.5 ± 16.5	54.8 ± 10.7†	918 ± 254	597 ± 140†	1,692 ± 349	2,163 ± 406†
Glucagon	400	116 ± 14.2	163 ± 21.9†	870 ± 204	2,755 ± 450†	2,067 ± 380	1,640 ± 453*

NOTE. Results are the mean ± SEM.
*$P < .02$, †$P < .01$: *v* basal.

Table 2. Maximum Response to Glicentin and Glucagon During Arginine Infusion in Six Dogs

Peptide	Dose (pmol)	IRI (mU/L)		IRG (ng/L)		Total IRG (ng/L)	
		Basal	Maximal	Basal	Maximal	Basal	Maximal
Glicentin	100	113 ± 27.3	266 ± 95.4*	1,082 ± 148	−167 ± 87.4	2,083 ± 349	1,442 ± 309‡
Glicentin	400	120 ± 45.5	270 ± 105*	1,481 ± 298	218 ± 60.3	2,372 ± 377	1,428 ± 307†
Glucagon	400	121 ± 39.7	414 ± 149*	1,407 ± 400	1,667 ± 456	2,753 ± 496	717 ± 321

NOTE. Results are the mean ± SEM.
*$P < .05$, †$P < .02$, ‡$P < .01$: *v* basal.

peak I of gut GLI with large molecular weight induced a tiny increase in plasma IRI in the pancreatic vein.[17] Furthermore, among the glicentin-related peptides, oxyntomodulin, glicentin(33-69), was observed to enhance insulin secretion, whereas neither glicentin(1-16) nor glicentin(62-69) stimulated insulin release.[18] In 1981, peak I of gut GLI was identified as glicentin(1-69),[1] but an insulinotropic action of glicentin(1-69) has not been clarified, although glucagon is contained in the sequence at amino acids 33 to 61.

In the present study, the insulinotropic action of glicentin was demonstrated for the first time. In the present experiment, recombinant human glicentin in two graded doses was administered into the PA to investigate a direct effect of glicentin on β-cell function. A previous study showed that plasma IRG in the PV reached approximately 1 nmol/L after administration of 400 pmol glucagon within 10 minutes into the PA.[19] According to another previous study, an oral load of glucose elicited an increase of plasma glicentin in the superior vena cava to nearly 1 nmol/L in pigs.[6] Therefore, it is assumed that the dosage of glicentin used in the present study induced a concentration of plasma glicentin within the physiological range.

The different results observed in the previous experiments concerning the insulin-releasing action of peak I of GLI or glicentin might derive from the smaller amounts of peptides administered. In the previous study in our laboratory, peak I of gut GLI was administered into the PA at a dose of 20 ngEq glucagon, which corresponds with 6 pmol glicentin.[17] To maintain the plasma concentration of 200 to 1,000 pmol/L glicentin in the PA, we should have administered the peptide at a dose of 80 to 400 pmol. Because the plasma concentration of glicentin in the PA might reach a level of 1 nmol/L after meals, the findings observed in the present study concerning the insulin-releasing action could be applied in physiological states.

In the present experiment, human glicentin was administered in an amount of 100 or 400 pmol within only 10 minutes into the PA during glucose infusion, and a significant but small increase of plasma IRI was observed in the PV. During infusion of 0.5% glucose, whole-blood glucose in the PV increased by approximately 10 mg/dL (data not shown). Following an oral glucose load, blood glucose in the peripheral vein increased to 160 mg/dL in a group of normal dogs in the conscious state.[20] Blood glucose levels in the PV during glucose infusion in the present study differ from those in the peripheral vein after an oral glucose load. The purpose of glucose infusion into the PA is to increase the sensitivity of β cells in response to glicentin. In a previous study with dogs,[21] plasma IRI in the PV increased from 150 to 1,430 μU/mL after an oral glucose load, whereas intravenous administration of glucose to simulate the hyperglycemic curve of the oral glucose load elicited an increase of plasma IRI in the PV from 180 to 962 μU/mL. The difference of the increase in plasma IRI in the PV could be explained as an incretin effect by gut factors released following an oral glucose load. At present, we have not established the assay system of human or canine glicentin, and it is not possible to measure plasma glicentin levels in man or dogs. However, according to a previous experiment with pigs,[6] plasma glicentin in the peripheral circulation increased to 881 pmol/L 30 minutes after an oral glucose load and remained elevated for 90 minutes or longer. In another previous study,[22] it was demonstrated that plasma total IRG in the peripheral vein reached a level of 613 pmol/L and stayed elevated for 60 minutes after an oral glucose load in the conscious state in normal dogs. Therefore, to assess the insulinotropic action of glicentin during an oral glucose load, the dosage of glicentin used in the present study was too small. Concerning the insulinotropic action of the intestinal extracts, small increases in plasma IRI in the PV were observed in the previous studies,[17,21] although small dosages of GLI were administered, as mentioned above. Therefore, to prove the insulinotropic action of glicentin, it is necessary to administer a large amount of glicentin comparable to that released from the gut during the maintenance of hyperglycemia observed following an oral glucose load.

In contrast, the insulin response to glicentin during arginine infusion was remarkable in the present study and comparable to that obtained following intraduodenal administration of an amino acids mixture, which elicited an increase in plasma IRI in the PV to 345 μU/mL in unanesthetized dogs.[23] The results obtained in the experiments during arginine infusion suggest that insulin release would be enhanced when a mixed meal containing carbohydrate and protein is administered.

In the present study, glicentin elicited the insulin-releasing action much more clearly during arginine infusion than during glucose infusion. The reason for the difference in the insulin response to glicentin during infusion of glucose and arginine is not fully explained. The same findings were observed when the *N*-terminal fragments of glucagon were administered into the canine pancreas.[24] Arginine stimulates glucagon secretion. Indeed, plasma glucagon in the PV is slightly higher during arginine

infusion. Therefore, it seems possible that the endogenous glucagon induced by the arginine infusion enhanced the insulin response to glicentin. Recently, it was reported that arginine increases depolarization of the membranes of pancreatic β cells and elevates the intracellular calcium concentration.[25] There is a possibility that glicentin stimulates insulin secretion through the intracellular transduction induced by arginine administration.

The precise mechanism through which glicentin stimulates insulin release is unknown at present. It seems likely that glicentin administered into the PA is modified in the pancreas into glucagon, which enhances insulin secretion. However, according to gel chromatography of plasma obtained from the PV (data not shown), it was suggested that glicentin administered into the PA passed through the pancreas without further processing. Therefore, glicentin itself would promote insulin release from the β cell of the pancreatic islet. The mechanism of the insulin-releasing action of glicentin remains to be elucidated in future studies.

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