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Effect of glicentin-related peptides on glucagon secretion in anaesthetized dogs

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Summary. Recent studies have demonstrated that glicentin is released during nutrient ingestion. However, the biological function of glicentin remains unclear. In order to clarify the role of glicentin in the enteroinsular axis, the effect of glicentin-related peptides was investigated using in vivo local circulation of canine pancreas. Peaks I and II of gut glucagon-like immunoreactivity, partially purified from porcine intestinal extract by affinity chromatography and gel filtration, synthesized hexadecapeptide of N-terminal glicentin (1-16) and synthesized octapeptide of C-terminal glicentin (62-69) were administered for 10 min into the pancreaticoduodenal artery of canine pancreas. Blood samples were then drawn from the pancreaticoduodenal vein. The administration of peak I of glucagon-like immunoreactivity during arginine infusion in a dosage of 20 ng reduced the glucagon secretion by 42 pmol/l ($p<0.05$), whereas peak II of glucagon-like immunoreactivity (20 ng) slightly increased the plasma level of insulin, although not significantly. The administration of glicentin (1-16) in a dosage of 400 ng during saline infusion did not alter the plasma insulin level, but reduced the plasma glucagon level in the pancreaticoduodenal vein by 29 pmol/l ($p<0.05$). In addition, glicentin [62-69] in a dosage of 400 ng exerted a decrease in both the plasma insulin (40 mU/l, $p<0.05$) and glucagon level (27 pmol/l, $p<0.05$). The present study demonstrates the suppression of pancreatic glucagon release during the infusion of peak I glucagon-like immunoreactivity and N- or C-terminal glicentin-related peptide. Therefore, it is suggested that glicentin released during nutrient intake might inhibit the secretion of glucagon.

Key words: GLI, glicentin-related peptide, glucagon, insulin, dog pancreas

Glicentin has been known not only as a component of gut glucagon-like immunoreactivity (GLI) [1], but also as a precursor of pancreatic glucagon [2, 3]. Recently, it has been proven that the secretion of glicentin is enhanced during the intraluminal administration of glucose or fat [4, 5]. According to our study, the plasma level of porcine glicentin is nearly 500 pmol/l at fasting and ranges to 2000 pmol/l during the ingestion of nutrients. Therefore, the plasma concentration of glicentin is relatively higher in comparison with other gastrointestinal hormones [5]. However, its biological action has not been established, although many aspects of glicentin activity have been reported by various authors [7-10]. The incretin effect of glicentin is the most interesting among them, because glicentin is released following nutrient ingestion. Therefore, the present experiment was performed in order to elucidate the effect of glicentin-related peptides upon the endocrine function of the pancreas in dogs.

Materials and methods

Materials

In the present study, four glicentin-related peptides were investigated. Peaks I and II of gut GLI were partially purified from the porcine intestinal crude extract by affinity chromatography and gel filtration as reported previously [8]. A typical pattern of gel filtration of GLI is shown in Figure 1. For the study of biological action, the central part of each peak was used. These original solutions of the peaks I and II contained 1470 and 2330 pmol/l of GLI measured by a non-specific antiserum to pancreatic glucagon (G-25) respectively. Peak I contained glicentin measured by glicentin antiserum (R-64) donated by Dr. A. J. Moody, Novo Institute, Copenhagen, Denmark. In contrast, no immunoreactive glicentin was detected in peak II. Only a minute amount of vasoactive intestinal peptide was measured in peak I

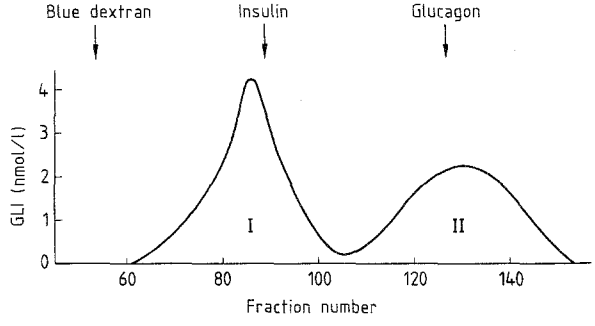

Fig. 1. Gel chromatography of porcine gut glucagon-like immunoreactivity (GLI) purified by immunoaffinity chromatography. The extract was applied to a column (2.6 × 70 cm, K 26/70) with BioGel P10 equilibrated by 50 mmol/l ammonium bicarbonate. Chromatography was performed at a rate of 26.4 ml/h and each fraction of 2 ml was collected. The column was calibrated by labelled insulin and glucagon. Immunoreactivity in each fraction was determined with an anti-glucagon rabbit serum (G 25), which reacts with gut GLI as well as pancreatic glucagon

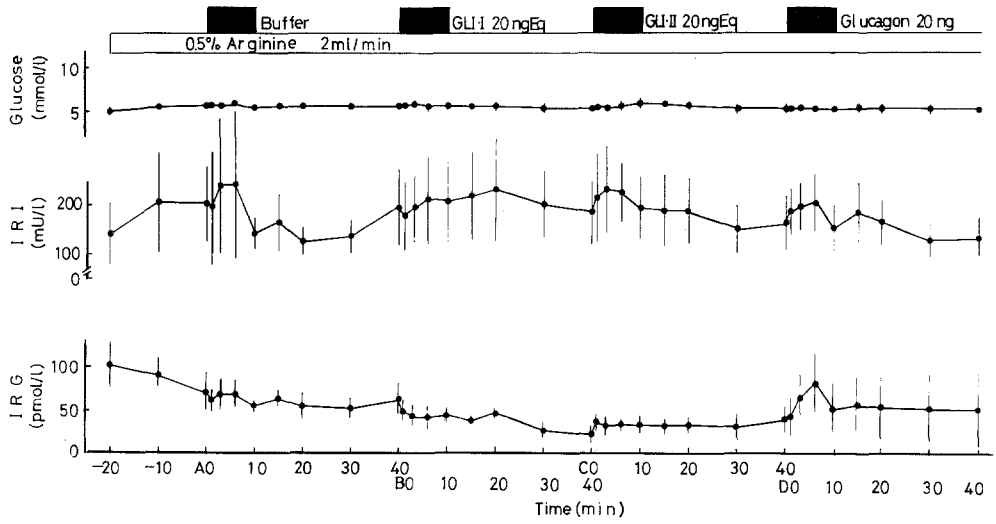

Fig. 2. Effect of peaks I and II of gut glucagon-like immunoreactivity (GLI) upon blood glucose in the femoral artery and plasma insulin and glucagon in the pancreaticoduodenal vein in a group of 5 anaesthetized dogs. Each value represents the mean ± SEM

(384 pg/ml) and peak II (80 pg/ml) by the courtesy of Otsuka Assay Laboratories, Tokushima, Japan. A hexadecapeptide of the N-terminal portion of glicentin (1-16) and an octapeptide of the C-terminal portion of glicentin (62-69) were donated by Dr. M. Fujino, Takeda Pharmaceutical Industries, Osaka, Japan. These peptides were prepared by the conventional solution method. The homogeneity of these peptides is confirmed by thin-layer chromatography, high performance liquid chromatography and amino acid analysis. Porcine crystalline glucagon (Lot. 258-25J-120) was donated by Eli Lilly Company, Indianapolis, Ind, USA.

Methods

In the present study, the canine pancreas was prepared for the in vivo local circulation as reported previously [11]. Healthy mongrel dogs weighing 12 to 16 kg were anaesthetized with pentobarbital sodium after an overnight fast, and the abdomen was opened by a midline incision. A glass T cannula connected to a teflon tube was inserted into the pancreaticoduodenal artery. A plastic needle was then inserted into the pancreaticoduodenal artery. Approximately 1 h after the completion of the operation, the test materials were infused into the pancreaticoduodenal artery and blood samples were obtained from the pancreaticoduodenal vein for hormone assay. In order to avoid adsorption of the test materials to the tubing, human albumin was added to each buffer solution at a concentration of 0.2%. For the hormone measurements, blood samples (4 ml) were obtained and collected into a glass tube containing 1000 KIU aprotinin (Trasylol, Bayer, Leverkusen, FRG) in ice. After the completion of the experiment, plasma was separated by centrifugation at 4 °C and stored at −20 °C until the assay. Plasma insulin (IRI) was measured by the immunoassay using the two antibody system [12]. Plasma glucagon (IRG) was measured by the immunoassay using antiserum (G 21) specific to the C-terminal portion of glucagon [13]. Blood samples for blood glucose were drawn from the femoral artery, and blood glucose was determined with the glucose oxidase method [14].

Statistical analysis

In the present experiment, the mean values and standard errors of the mean were calculated. Statistical analyses were performed by the Student's t-test for paired data. Confidence limits were set at $p < 0.05$.

Results

Figure 2 shows the effects of the partly purified gut GLI on the plasma levels of insulin and glucagon in the pancreaticoduodenal vein in a group of 5 dogs. During the constant infusion of 0.5% arginine solution at a rate of 2 ml/min into the pancreaticoduodenal artery, buffer solution (ammonium bicarbonate), peak I, peak II and pancreatic glucagon were infused for 10 min successively at 40 min intervals. Blood glucose was 4.9 ± 0.4 mmol/l at the baseline and increased to 5.8 ± 0.2 mmol/l 20 min after the arginine infusion. Blood glucose did not fluctuate following the buffer infusion. Plasma IRI increased from the fasting level of 144 ± 61 mU/l to a level of 204 ± 77 mU/l 20 min after the arginine infusion ($p < 0.05$). However, the buffer infusion did not elicit any changes in plasma IRI. Plasma IRG revealed no changes following either the arginine infusion or the buffer injection. Blood glucose was not altered by the administration of 20 ng of peak I of GLI. Plasma IRI was 194 ± 76 mU/l at the preinfusion period, and did not exert any significant changes after the infusion of peak I. Plasma IRG was 61 ± 18 pmol/l at the preinfusion period and slightly decreased to 35 ± 4.6 pmol/l at 15 min. Following the infusion of 20 ng of the peak II of GLI, blood glucose was slightly increased from the preinfusion level of 5.3 ± 0.3 mmol/l to 6.0 ± 0.2 mmol/l at 15 min ($p < 0.05$). Plasma IRI was slightly increased from the preinfusion level of 182 ± 64 mU/l to a peak of 234 ± 96 mU/l 3 min after the infusion of peak II, although the change did not reach significance. Plasma IRG was slightly elevated following the peak II infusion, although it was not statistically significant. Blood glucose did not change following the infusion of 20 ng of pancreatic glucagon. Plasma IRI was increased slightly from 142 ± 41 mU/l of the preinfusion level to 209 ± 59 mU/l 6 min after glucagon infusion. Plasma IRG was slightly increased to a peak of 81 ± 33.7 pmol/l 6 min following the glucagon infusion.

Figure 3 shows the changes in blood glucose, plasma IRI and IRG during the administration of the hexadecapeptide of glicentin. In this experiment, phosphate buffer, 400 ng of glicentin (1-16) and 200 ng of pancreatic glucagon were successively infused for 10 min during saline infusion in a group of 5 dogs. Blood glucose was 5.7 ± 0.2 mmol/l at fasting, and did

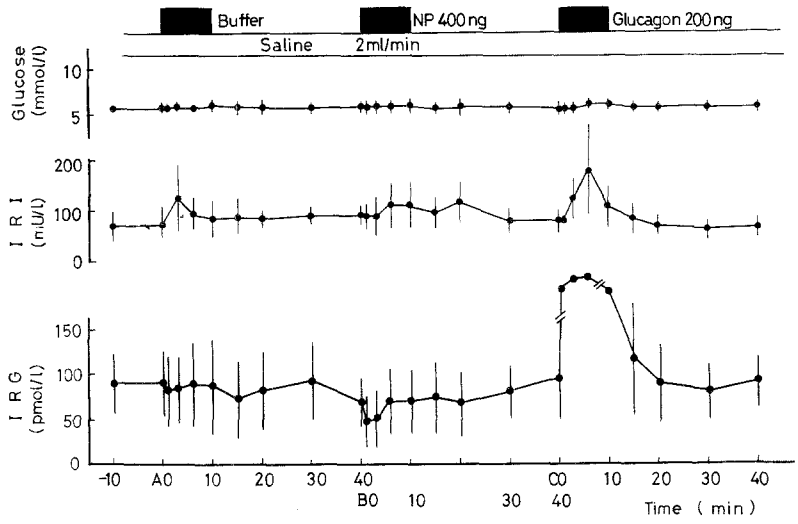

Fig. 3. Effect of hexadecapeptide of glicentin 1-16 (NP) upon blood glucose in the femoral artery and plasma insulin and glucagon in the pancreaticoduodenal vein in a group of 5 anaesthetized dogs. Each value represents the mean ± SEM

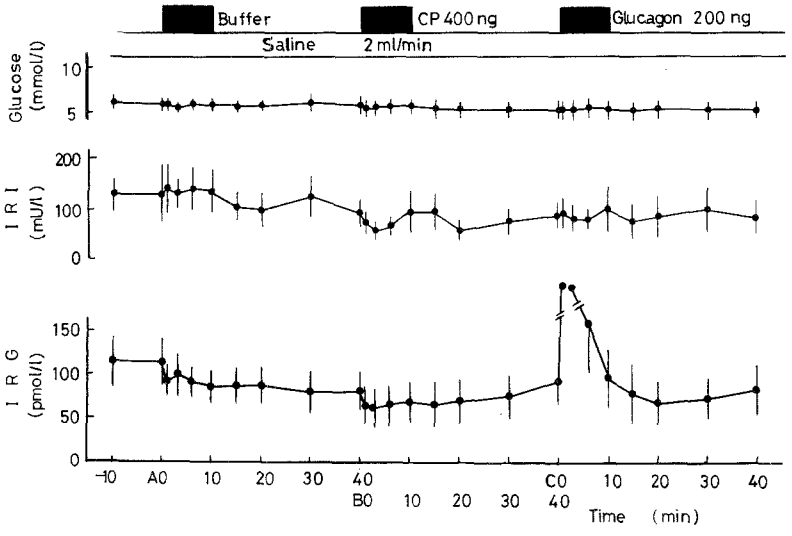

Fig. 4. Effect of octapeptide of glicentin 62-69 (CP) upon blood glucose in the femoral artery and plasma insulin and glucagon in the pancreaticoduodenal vein in a group of 5 anaesthetized dogs. Each value represents the mean ± SEM

not change after the buffer infusion. Plasma IRI and IRG were 69 ± 30 mU/l and 91 ± 36.9 pmol/l at the initial state respectively, and were not altered significantly following the buffer administration. Following the administration of glicentin (1-16), blood glucose did not change from the preinfusion level of 6.1 ± 0.7 mmol/l. Plasma IRI increased slightly after glicentin (1-16) infusion from the baseline level of 92 ± 18 mU/l, although not significantly. Plasma IRG was 68 ± 26.9 pmol/l at the preinfusion state and slightly decreased to a level of 50 ± 34.3 pmol/l 3 min after the infusion of glicentin (1-16). As a control, 200 ng of pancreatic glucagon was infused at the end of the experiment. After glucagon administration, blood glucose rose slightly. Plasma IRI was 78 ± 22 mU/l at the preinfusion state and increased to a level of 184 ± 89 mU/l at 6 min, although, due to wide deviations, not significantly. Plasma IRG was 95 ± 43.1 pmol/l at the preinfusion state, and increased promptly to a level of 287 ± 60 pmol/l at 3 min ($p < 0.05$).

The effects of glicentin (62-69) are presented in Figure 4. In this experiment, phosphate buffer, 400 ng of glicentin (62-69) and 200 ng of pancreatic glucagon were successively administered for 10 min during saline infusion at 40 min intervals in a group of 5 dogs. Blood glucose, plasma IRI and IRG were 6.1 ± 0.7 mmol/l, 129 ± 31 mU/l and 114 ± 26.3 pmol/l respectively, and did not change after the buffer infusion. Blood glucose was 5.6 ± 0.9 mmol/l at the preinfusion level, and did not reveal any significant change after the infusion of octapeptide of glicentin (62-69). Plasma IRI was 95 ± 25 mU/l at the preinfusion level, and slightly decreased following the infusion of the octapeptide. Plasma IRG was 81 ± 21.4 pmol/l at the initial state, and slightly decreased to 61 ± 23.1 pmol/l 3 min after the glicentin (62-69) infusion. After the administration of glucagon (200 ng) at the end of the experiment, blood glucose rose slightly from the baseline of 5.4 ± 1.0 mmol/l to 5.6 ± 0.9 mmol/l. Plasma IRI was 85 ± 27 mU/l at the preinfusion state, and slightly increased to a level of 100 ± 44 mU/l at 10 min. Indeed, plasma IRI rose after glucagon infusion in each dog. However, because the increment and the time of peak varied, the rise in plasma IRI after the glucagon infusion did not reach signifi-

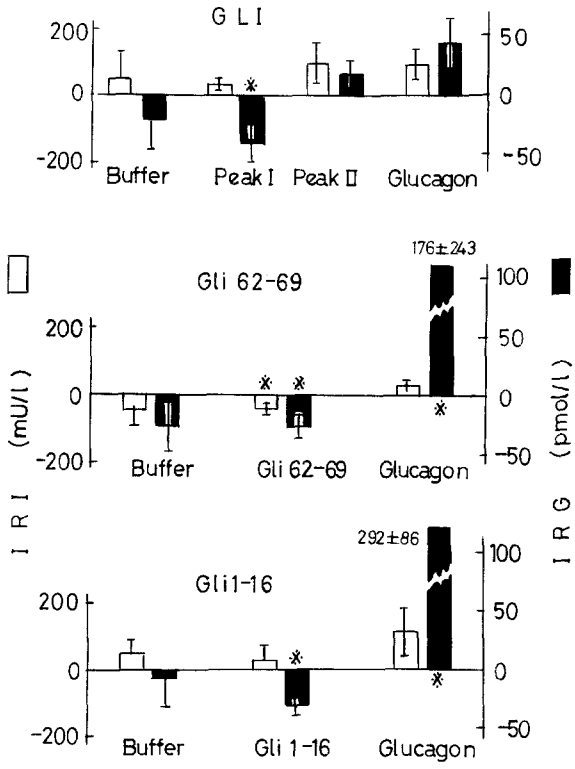

Fig. 5. Maximum response of plasma insulin (IRI) and glucagon (IRG) within 20 min to administration of peaks I and II of gut glucagon-like immunoreactivity (GLI), hexadecapeptide of glicentin (GLi 1-16) and octapeptide of glicentin (GLi 62-69). The asterisks represent a significant difference ($p < 0.05$)

cance. Plasma IRG was 91 ± 37.1 pmol/l and reached a level of 263 ± 90.3 pmol/l at 1 min.

In order to compare the effects of glicentin-related peptides on the endocrine function of the pancreas, the maximum change of plasma IRI and IRG within 20 min were calculated, as shown in Figure 5. As far as GLIs are concerned, peak I significantly reduced the plasma IRG in the pancreatic vein ($p < 0.05$), whereas peak II slightly increased plasma IRI. The hexadecapeptide of the N-terminal portion of glicentin revealed a decrease in plasma IRG ($p < 0.05$). The octapeptide of the C-terminal portion of glicentin induced a decrease in both the plasma IRI and IRG ($p < 0.05$). Glucagon administered at the end of each experiment exerted a slight increase in plasma insulin concurrently with the increase in plasma glucagon levels in response to each dosage.

Discussion

In the present study, gut GLI was partly purified from crude extract of porcine intestine by affinity chromatography and gel filtration as reported previously [8]. Further, in order to investigate the changes in plasma glucagon, 0.5% solution of arginine was infused into the pancreaticoduodenal artery throughout the experiment instead of glucose, because the elevation of plasma glucose may suppress glucagon secretion. As shown in Figures 2 and 5, plasma insulin in the pancreaticoduodenal vein was increased during the infusion of peak II and glucagon, but not during the infusion of peak I, confirming the previous study [8]. In contrast, it was demonstrated that the secretion of pancreatic glucagon was reduced during the administration of peak I of GLI.

Furthermore, to investigate the effect of glicentin-related peptides upon the endocrine function of the pancreas, two glicentin-related peptides were infused in other experiments. As shown in Figures 3 and 4, both the N- and C-terminal peptides of glicentin exerted a decrease in plasma glucagon in the pancreaticoduodenal vein. Kawai and Unger [17] reported that the secretion of pancreatic glucagon is suppressed by exogenous glucagon. It is well known that glicentin contains a glucagon molecule [1]. Therefore, the suppression of pancreatic glucagon during the infusion of peak I of GLI could be attributed to the effect of glucagon in the glicentin molecule. In the present study, however, glicentin-related peptides, C- and N-terminal portion of glicentin, also exerted a decrease in glucagon secretion from the pancreas. Therefore, it is reasonable to consider that suppression of pancreatic glucagon during peak I infusion might be induced by both the glucagon and glicentin-related peptides. From the knowledge on the processing of glicentin from the precursor of the hormone, glicentin related pancreatic peptide (GRPP, glicentin 1-30) and oxyntomodulin (glicentin 32-69) are important. Therefore, the effect of these peptides should be investigated in future.

The dosage of glicentin-related peptides should be considered. In the experiment with porcine GLI peaks, 20 ng equivalent to glucagon was administered as previously reported [8]. Calculation of the glucagon level in the pancreaticoduodenal artery based on the plasma flow indicates that the dosage of each GLI peak brings about the increase in the plasma GLI level within the physiological range. In contrast to GLI experiment, however, we infused 400 ng of N- or C-terminal peptide of glicentin, corresponding to 216 or 418 pmol respectively. According to the calculation based on the blood flow of the pancreaticoduodenal artery, these dosages would exert the plasma level in the pancreaticoduodenal artery of 2.16 or 4.18 nmol/l for each peptide, which might be beyond the physiological range. Therefore, the effect of the glicentin-related peptides upon the glucagon level in the pancreaticoduodenal vein could be presumed as a pharmacological one.

Gut GLI extracted from the intestine can be divided into two peaks, peaks I and II, which contain glicentin and oxyntomodulin respectively [1, 18]. However, chromatography of circulating plasma GLI following nutrient ingestion demonstrated that a large part of the GLI consists of glicentin, while oxyntomudoulin is observed as a small peak at least in the peripheral blood [4, 5]. Therefore, glicentin might play an important role in

the enteroinsular axis, although peak II enhances insulin secretion.

Physiological significance of glucagon suppression by glicentin is at present unknown. It is well recognized that glucagon secretion is decreased following oral glucose administration. Since glicentin is released after glucose ingestion [4], suppression of glucagon secretion could be attributed to hyperglycaemia as well as increased secretion of glicentin. In a group of patients with postprandial hypoglycaemia, gut GLI is released much more after meal ingestion [19]. It has been reported that glicentin does not displace 125-I glucagon bound to liver plasma membrane [20]. Therefore, it is possible that the elevated GLI level following carbohydrate ingestion might promote hypoglycaemia by the suppression of glucagon secretion in the pancreas. Further investigation should be performed using purified glicentin and its related peptides, such as GRPP or oxyntomodulin, in order to determine the biological function of glicentin in the endocrine pancreas.

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