Insulin Releasing Action of N-Terminal Peptides of Glucagon in Dogs

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Ohneda, A., Ohneda, K. and Koizumi, F. Insulin Releasing Action of N-Terminal Peptides of Glucagon in Dogs. Tohoku J. Exp. Med., 1992, 167 (4), 287-295 — The relationship between the molecular structure and insulin releasing action of glucagon remains unknown. In order to investigate the direct action of N-terminal peptides of glucagon, glucagon (1-14), and glucagon (1-21) were studied using an in situ local circulation of the canine pancreas. These glucagon fragments as well as glucagon (1-29) were infused into the superior pancreaticoduodenal artery in a dose of 400 pmol for 10 min during the glucose or arginine infusion, and plasma insulin and glucagon in the superior pancreaticoduodenal vein were determined by radioimmunoassay. During the glucose infusion, glucagon (1-14) elicited a slight increase in plasma insulin, whereas glucagon (1-21) and (1-29) revealed significant changes in plasma insulin. In these experiments plasma glucagon did not change significantly following the administration of glucagon (1-14) or (1-21). During the arginine infusion all of the glucagon fragments studied enhanced insulin secretion markedly, whereas glucagon secretion was not affected. Furthermore, graded doses of glucagon (1-14) (50, 150, and 400 pmol) elicited an increase in plasma insulin in a dose-related manner. It is concluded from the present study that the N-terminal peptides of glucagon stimulate insulin release especially during the arginine infusion.

It has been well known that glucagon enhances insulin secretion since the first report by Samols et al. (1965). However, which parts of the glucagon molecule have an effect on insulin secretion has remained unknown. Assan and Slusher reported the relationship of the molecular structure and function of glucagon (Assan and Slusher 1972). According to their finding, none of several fragments of glucagon, as far as they studied, revealed an insulin stimulating action in man. Therefore, they concluded that the whole molecule of glucagon was necessary to elicit an enhancement of insulin secretion. During the study concerning the effect of glucagon-like peptides 1 (GLP-1), we observed an enhancement of insulin secretion with GLP-1 (7-20) (Ohneda et al. 1991). Since GLP-1 (7-20) has similarities in 71% in the amino acid sequence to that of glucagon (1-14), this fact
prompted us to investigate the relationship between the molecular structure and insulin releasing action of glucagon fragments. Therefore, the present study was carried out to investigate the direct effect of N-terminal peptides of glucagon upon the B cell function of the canine pancreas.

**Materials and Methods**

In the present study, N-terminal fragments of glucagon, (1-14) and (1-21), were investigated using an in situ local circulation system of the canine pancreas, as reported previously (Ohneda et al. 1977). Synthesized glucagon (1-14) was given by Dr. Fujino (Takeda Chemical Industries, Osaka). The analytical data of the peptide was reported elsewhere (Ohneda et al. 1979). Glucagon (1-21) and porcine glucagon were supplied by Novo Institute (Bagsvaerd, Denmark) and Eli Lilly Co. (Indianapolis, IN, USA), respectively.

In the present experiments, 31 healthy mongrel dogs weighing 10 to 14 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 to 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 (FA). Approximately one hour after the operation the experiments were started. After the initial samples were drawn, 0.5% glucose or 0.5% arginine solution (Morishita Pharmaceutical Co., Tokyo) was infused into the PA at a rate of 2 ml/min. During glucose or arginine infusion, the glucagon fragments were administered into the PA in a dosage of 400 pmol within 10 min. In order to further investigate the effect of glucagon (1-14) upon endocrine function of the pancreas, graded doses of the peptide, 50, 150 and 400 pmol, were administered successively into the PA during the arginine infusion. In order to avoid adsorption of the test materials to the tubing, bovine serum albumin (Rehis Chemical Co., Phoenix, AZ, USA) was added to each solution at a concentration of 0.2%. Blood samples were obtained 10 min and just before and 1, 3, 6, 10, 15, 20, 30 and 40 min after the start of the peptide infusion. For the hormone assay blood was drawn from the PV in amount of 4 ml and collected into a glass tube containing 1,000 KIU of aprotinin (Trasylol, Bayer Co., Leverkusen, Germany) and 10 mg EDTA in ice. After the 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 FA.

Plasma insulin (IRI) was determined by immunoassay with the two-antibody system (Morgan and Lazarow 1963). Plasma glucagon (IRG) was measured by immunoassay using an antiserum G21, specific to the C-terminal portion of glucagon, as reported elsewhere (Ohneda et al. 1975). For the hormone assay porcine insulin, donated by Eli Lilly Co., Indianapolis, IN, USA, and porcine glucagon were used as the standard materials. Blood glucose was determined with the glucose oxidase method.

For a comparison of the effect of the glucagon fragments upon the endocrine function of the pancreas, the maximal changes for 20 min following the infusion of each peptide in plasma IRI and IRG were calculated.

In the present study, the mean ± s.e. was calculated. Statistical analyses of the changes in blood glucose, plasma IRI and IRG were performed with analysis of variance or Friedman test when applicable. For a comparison of the maximal changes in plasma IRI and IRG, statistical analyses were carried out with Student's t-test.

**Results**

*The effect of glucagon fragments during glucose infusion*

During the glucose infusion, glucagon (1-14) and glucagon (1-21) were
Insulin Relaeesing Action of Glucagon Fragments

successively administered at a 40-min interval to a group of 6 dogs (Fig. 1). Blood glucose did not change significantly following the infusion of these glucagon fragments. The administration of glucagon (1–14) elicited a slight increase in plasma IRI, although not significantly. Following the infusion of glucagon (1–21) plasma IRI increased from the base line level of 2.06±0.91 to a peak of 3.30±1.59 nM at 6 min (p < 0.01). Plasma IRG did not change significantly following the infusion of glucagon (1–14) or (1–21). For a comparison the data obtained in another group of 6 dogs, administered glucagon (1–29) during the glucose infusion, were depicted in Fig. 1. Blood glucose rose gradually from the initial level of 6.8±0.8 to a peak of 8.0±1.1 mM at 20 min (p < 0.01). Plasma IRI slightly increased from 0.90±0.16 to a peak of 1.48±0.43 nM 3 min after the glucagon infusion (p < 0.05). Plasma IRG reached a peak of 3,867±95 ng/liter at 6 min (p < 0.01).

The effect of glucagon fragments during arginine infusion

During the arginine infusion, glucagon (1–14) and (1–21) were administered successively at a 40-min interval to a group of 7 dogs (Fig. 2). Blood glucose did not show any changes throughout the experiment. Following the infusion of glucagon (1–14) plasma IRI rose significantly from the initial level of 0.55±0.21 to a peak of 1.55±0.73 nM at 3 min (p < 0.01). The infusion of glucagon (1–21) elicited a rise in plasma IRI from 0.35±0.19 to a peak of 1.39±0.49 nM at 1 min.

Fig. 1. Changes in blood glucose and plasma insulin and glucagon in response to the administration of glucagon (1–14) (A), glucagon (1–21) (B), and glucagon (1–29) (C) into the superior pancreaticoduodenal artery of the pancreas during the glucose infusion in each group of 6 dogs. Values are the mean±s.e.
Plasma IRG did not reveal any changes following the infusion of each of these glucagon fragments. For a comparison, the changes in blood glucose, plasma IRI and IRG following the glucagon (1–29) infusion to another group of 5 dogs were depicted in Fig. 2. Blood glucose rose from the base line of $5.1 \pm 0.3$ to a peak of $6.7 \pm 0.7$ nM 15 min after the glucagon (1–29) infusion ($p < 0.01$). Plasma IRI increased from the base line of $1.00 \pm 0.47$ to a peak of $2.64 \pm 1.37$ nM at 6 min ($p < 0.01$). Plasma IRG reached a peak of $3,010 \pm 607$ ng/liter following the glucagon (1–29) infusion ($p < 0.01$).

The effect of graded doses of glucagon (1–14) during arginine infusion

During the arginine infusion, graded doses of glucagon (1–14) were administered successively at 40-min intervals to a group of 7 dogs (Fig. 3). Blood glucose did not change significantly following the administration of 50 pmol of glucagon (1–14). After the infusion of 50 pmol of glucagon (1–14) plasma IRI rose significantly from the base line level of $1.19 \pm 0.62$ nM to a peak of $2.14 \pm 0.28$ nM at 1 min ($p < 0.01$). Plasma IRG did not change significantly following the administration of 50 pmol of glucagon (1–14) (Fig. 3A).

When 150 pmol of glucagon (1–14) was administered during the arginine infusion, blood glucose slightly but significantly decreased from the base line level

![Fig. 2. Changes in blood glucose and plasma insulin and glucagon in response to the administration of glucagon (1–14) (A), glucagon (1–21) (B) and glucagon (1–29) (C) into the superior pancreaticoduodenal artery of the pancreas during the arginine infusion. The experiments A and B were carried out in a group of 6 dogs, while the experiment C in another group of 5 dogs. Values are the mean ± s.e.](image-url)
of 7.3±1.10 to 6.6±1.03 mM at 20 min (p <0.01). Following the administration of 150 pmol of glucagon (1-14), plasma IRI rose significantly from the preinfusion level of 1.76±0.61 to a peak of 3.08±0.89 nM at 1 min (p <0.01). After the administration of 150 pmol of glucagon (1-14) plasma IRG did not change significantly (Fig. 3B).

The administration of 400 pmol of glucagon (1-14) elicited a significant decrease in blood glucose from the base line level of 6.7±0.92 to a nadir of 6.3±0.92 mM at 20 min (p <0.01). Plasma IRI increased from the preinfusion level of 1.15±0.32 to a peak of 2.76±0.88 nM 3 min after the administration of 400 pmol of glucagon (1-14) (p <0.01). Following the administration of 400 pmol of glucagon (1-14) plasma IRG did not change significantly (Fig. 3C).

**Comparison of the responses in plasma insulin and glucagon to glucagon fragments**

The maximal changes of plasma IRI and IRG in response to these glucagon fragments during glucose or arginine infusion are presented in Table 1. During the glucose infusion, the administration of glucagon (1-14) elicited a significant change in plasma IRI (p <0.01), whereas glucagon (1-21) showed an increase with a wide deviation in plasma IRI. The administration of glucagon (1-29) elicited a significant increase in plasma IRI (p <0.01). The maximal changes in plasma IRG were not significant following the administration of glucagon (1-14) or glucagon (1-21). The administration of glucagon (1-29) revealed a large increase in plasma IRG (p <0.01).

During the arginine infusion, glucagon (1-14), (1-21) and (1-29) elicited a
The administration of glucagon (1-14) and glucagon (1-21) elicited significant maximal increases in plasma IRG, 426 and 689 ng/liter, respectively (p < 0.01). The administration of glucagon (1-29) showed a large increase in plasma IRG (p < 0.01).

During the arginine infusion all the graded doses of glucagon (1-14) revealed a significant increase in plasma IRI in a dose-related manner (p < 0.05 or less). The administration of 50 or 150 pmol of glucagon (1-14) elicited a significant increase in plasma IRG (p < 0.01).

The calculation of the increment area of plasma IRI during 20 min following the infusion of each peptide revealed an almost same tendency as the maximal changes.

**DISCUSSION**

Since 10 among 14 amino acids in the sequence of glucagon (1-14) are similar to that of GLP-1 (7-20), glucagon (1-14) was expected to reveal an insulin releasing action, from the previous study (Ohneda et al. 1991). This was proved in the present study. Furthermore, glucagon fragment (1-21) also enhanced insulin secretion.

There have been few reports, which deal with an insulin releasing action of the glucagon-related peptides. Assan did not observe any hyperglycemic action nor insulin releasing action of glucagon fragments (1-23) and (9-23) in man (Assan and Slusher 1972). An intravenous administration of glucagon (1-21) did
not elicit any changes in blood glucose and plasma insulin in normal subjects (Owens, D.R., personal communication). It was reported that glucagon fragment (1-21) or (22-29) did not reveal any changes in the insulin level in a perfusion experiment of the isolated rat pancreas (Komatsu et al. 1988).

In our experiments, an in situ local circulation system of the canine pancreas was employed in order to investigate the direct effects of several glucagon fragments upon the endocrine function of the pancreas. From the calculation of the dosage of these glucagon fragments based on the blood flow of the pancreas, 400 pmol of each peptide was administered into the PA for 10 min in order to maintain 1 nM, as reported previously (Ohneda et al. 1977). As shown in the results with glucagon (1-29), the expected concentration of these glucagon fragments was thought to be achieved in the PV. The different results obtained in the present study concerning the insulin releasing action might be explained by the different dosage used and different animal species, comparing the findings reported hitherto (Assan and Slusher 1972; Komatsu et al. 1988).

The present study shows that glucagon (1-14) and glucagon (1-21) elicited much more clearly the insulin releasing action during the arginine infusion, in comparison with that during the glucose infusion. The result suggests that these glucagon fragments rather stimulate arginine-induced insulin secretion. The reason for the difference in the insulin response to the glucagon fragments during the infusion of arginine and glucose is not fully explained at present. The glucose infusion suppresses glucagon secretion, whereas the arginine infusion stimulates glucagon secretion. Indeed the concentration of plasma IRG in the PV was higher during the arginine infusion than the glucose infusion. Therefore, it seems possible that endogenous glucagon secretion induced by the arginine infusion enhanced the insulin response to glucagon fragments.

In the present study, N-terminal glucagon fragments, (1-14) and (1-21), elicited the almost similar effect on insulin secretion to that with glucagon (1-29). The finding suggests that tetradecapeptide (1-14) of the N-terminus of glucagon is a smaller structure of glucagon to stimulate insulin secretion.

It has been reported that some truncated GLP-1 peptides elicit an enhancement of insulin secretion and an inhibition of glucagon secretion (Kreymann et al. 1987; Matsuyama et al. 1988; Ørskov et al. 1988; Kawai et al. 1989). In the present experiments, glucagon secretion was affected by neither glucagon (1-14) nor glucagon (1-21), which enhanced insulin secretion. Therefore, it seems that glucagon (1-14) and (1-21) do not reveal any glucagon suppression through the increase in insulin secretion with glucagon (1-14) or glucagon (1-21). Therefore, the present study suggests that the N-terminal fragments of glucagon affect mainly the B cells of the pancreatic islet.

In the present study, two N-terminal fragments of glucagon were employed, based on the interest in the relationship between the molecular structure and function of glucagon. Since glucagon includes a dibasic pair at 17th and 18th
The precise mechanism through which insulin secretion is promoted with these glucagon fragments is unknown at present. None of these peptides revealed the elevation of blood glucose, suggesting that they do not affect an increase in the intracellular cyclic AMP level in the liver. The N-terminal His of the glucagon molecule is considered important in the binding and action of glucagon (Rodbell et al. 1971). However, a discrepancy was reported between the receptor binding and biological action of replacement analogs of glucagon in the rat hepatocyte membrane (Unson et al. 1991). In this context, glucagon (1-21) does not displace labelled glucagon bound to the plasma membrane of the hepatocyte nor activate adenylcyclase in the liver (Frandsen et al. 1985). Nevertheless glucagon (1-21) also stimulates insulin release. Therefore, glucagon (1-14) and (1-21) might reveal an insulin releasing action through a different way other than glucagon receptor. The mechanism of the insulin releasing action with glucagon fragments remains to be clarified in future.

References


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