The Essential Amino Acid Domains in Salivary Peptide P-C That Potentiate Glucose-Induced Insulin Release and Inhibit Arginine-Induced Glucagon Release from Perfused Rat Pancreas

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ABSTRACT—The amino acid domains in salivary peptide P-C (1-44 peptide fragments, P-C) that are essential to potentiate insulin release and inhibit glucagon release were investigated using isolated perfused rat pancreas. P-C significantly potentiated not only glucose (8.3 mM)-induced insulin release, but also arginine (10 mM)-induced insulin release. The essential domain responsible for potentiation of insulin release was the P-C-(23-29) fragment, KPQGPPP, while that inhibiting glucagon release was the P-C-(12-18) fragment, HQQGP. Since both domains share a common fragment, QGPP, these findings indicate that the functional amino acid sequences KP and HQ may potentiate insulin release and inhibit glucagon release, respectively.

Keywords: Amino acid domain (salivary peptide P-C), Insulin release, Glucagon release

The salivary peptide P-C (P-C) isolated from human saliva is a proline-rich peptide consisting of 44 amino acid residues (1). From immunohistochemical studies, P-C-like immunoreactivity was found to be localized not only in the serous cells of the salivary gland, but also in human pancreatic B cells (2), namely in the insulin-secretory granules of pancreatic B cells (3). We have reported that P-C remarkably potentiates glucose (8.3 and 16.7 mM)-induced insulin release, whereas the same concentration inhibits arginine (10 mM)-induced glucagon release in a concentration-dependent manner (4). Glucagon-like peptide 1 (GLP-1) stimulates insulin release and decreases glucagon release (5) similar to P-C, but it is noteworthy that the constitutional amino acid sequences in GLP-1 are quite different from those in P-C. The present study was conducted to investigate the amino acid domains in P-C involved in potentiating glucose (8.3 mM)- and arginine (10 mM)-induced insulin release and those responsible for inhibiting arginine (10 mM)-induced glucagon release.

Male Wistar rats, 6- to 7-weeks-old (weight, 180-300 g), were anesthetized with sodium pentobarbital (Nembutal, 50 mg/kg, i.p.; Abbott Lab., North Chicago, IL, USA), and then the pancreas was isolated along with the stomach, duodenum and spleen in order to keep the pancreas intact, as previously described in detail (4). Perfusion was carried out through the celiac artery with a basal medium of Krebs-Ringer bicarbonate buffer solution (pH 7.4) containing 0.5% bovine serum albumin (Fraction V; Sigma, St. Louis, MO, USA), 2% dextran (T-70; Pharmacia, Uppsala, Sweden) and 2.8 mM D-glucose (Wako, Osaka). The Krebs-Ringer medium was saturated with a gas mixture of 95% O2 and 5% CO2. Salivary peptide P-C-(1-44, H-GRPQGPPQQGHQGPPPPPQKPQGPPPQGPPQQGPPQGQSPQ-OH) and its fragment peptides: P-C-(1-22, H-1GRPQGPPQQGHQGPPPPPQK-QPQGPPPQGPPQGQSPQ-OH); P-C-(23-44, H-KPQGPPPPPQGGRPPQPPPQGQPQ-OH); P-C-(18-12, H-HQQGP-QPQGPPPQGPPPPP-OH); P-C-(H-HQQGP-PP-OH); and P-C-(H-KPQGPPP-OH) were chemically synthesized by Teikoku Hormone Mfg. Co. (Tokyo). Arginine HCl (L-isomer, Wako) was also used.

The concentrations of insulin and glucagon contained in samples that were collected from a portal vein catheter were...
determined by radioimmunoassay as reported previously (6). The insulin-releasing effect was defined as the ratio of the concentrations in the presence and absence of P-C fragments. Data are expressed as means ± S.E.M. The paired and unpaired t-test were used to determine statistical significance at a P-level of 0.05 or 0.01.

We investigated the amino acid residues in P-C involved in potentiating the glucose (8.3 mM)-induced increase in immunoreactive insulin (IRI) levels during a 10-min period in the isolated perfused rat pancreas. Glucose was added three times every 10 min to the perfused pancreas preparation. The additions were followed by 10-min washing with Krebs-Ringer medium. With this experimental design, we were able to accomplish a constant release of IRI in response to repeated glucose (8.3 mM)-stimulation. The lack of a priming effect of glucose on the pancreatic B cells in our results may be caused by the experimental conditions (basal and high glucose concentrations: 2.8 and 8.3 mM, flow rate of perfusion: 1.0 ml/min and washing time: 10 min), which were different from those in the previous report (7). The glucose-induced insulin release was defined as the ratio of insulin released in the presence and absence of P-C fragments (194 nM). The P-C-(23–29) fragment, 23KPQGPPP29, was the most potent in inducing IRI release among P-C and its various fragments at 194 nM (Fig. 1 and Table 1). Between the fragments composing P-C-(23–29), the P-C-(25–28) fragment, 25QGPP28, was the most potent in releasing insulin, but it was still less potent than P-C-(23–29).

P-C also potentiated the arginine (10 mM)-induced increase in IRI levels in the isolated perfused rat pancreas. Insulin-releasing effects were continuously measured for 30 min after the administration of 194 nM P-C with 10 mM arginine (Fig. 2), because arginine releases insulin on a single addition only (when the perfused pancreas was subsequentially stimulated with arginine, the second stimulation with arginine (10 mM) induced over an 80% inhibition of the insulin response to the first arginine stimulation). P-C potentiated both the first and second phases of the arginine-induced increase in IRI levels. The total amount of IRI release with P-C was significantly greater than that when arginine was given alone. P-C potentiated both glucose- and arginine-induced insulin release. We further investigated the essential amino acid domains in P-C that potentiated arginine (10 mM)-induced IRI release during the 30-min period. The P-C-(23–29), P-C-(19–31), P-C-(23–44), and P-C-(12–29) fragments showed similar potentiation of arginine-induced insulin release. These effects were slightly weaker than those of P-C (Table 1).

Furthermore, we investigated the essential amino acid domains in P-C responsible for inhibiting the arginine (10 mM)-induced release in immunoreactive glucagon (IRG) levels during a 10-min period. Arginine was added to the perfused pancreas three times during a 10-min period. The additions were followed by 10-min washing with Krebs-Ringer medium. In this experimental design, we accomplished a constant release of IRG in response to repeated arginine (10 mM)-stimulation. The arginine-induced glucagon release was defined as the ratio of glucagon released in the presence of P-C fragments (194 nM) to glucagon release with arginine alone. The P-C-(12–18) fragment, 12HQGQPP28, produced the strongest inhibition of arginine-induced IRG release among P-C and its fragments (Table 1). The inhibiting effect of P-C-(14–17), 14QGPP17, was the most potent of the fragments comprising the P-C-(12–18) fragment, but it was less potent than the P-C-(12–18) fragment. These results indicate that two separate functional centers in P-C are responsible for potentiating IRI release and inhibiting IRG release, respectively. Moreover, both of the essential domains in P-C...
Table 1. Salivary peptide P-C fragments and their potentiation of glucose (8.3 mM) and arginine (10 mM)-induced insulin release and inhibition of arginine (10 mM)-induced glucagon release in perfused rat pancreas

| P-C fragments | Insulin release | Glucagon release |
|---------------|----------------|-----------------|
|               | Release ratio a) |                  |
| P-C-(1–44)    | 1.64±0.04** 3) (10) | 0.76±0.03** 4) (9) |
| P-C-(1–22)    | 1.43±0.04** (5) | 0.76±0.02** (6) |
| P-C-(23–44)   | 1.71±0.10** (5) | 0.86±0.03** (5) |
| P-C-(19–31)   | 1.57±0.06** (5) | 0.87±0.03** (5) |
| P-C-(12–29)   | 1.78±0.09** (6) | 0.77±0.03** (5) |
| P-C-(12–18)   | 1.56±0.06** (5) | 0.73±0.02** (7) |
| P-C-(23–29)   | 2.01±0.07** (7) | 0.93±0.02** (5) |

*: Release ratio is defined as the ratio of release in the presence and absence of P-C fragments (194 nM) during a 10 min period (except for the absolute values during the 30 min period in arginine-induced insulin release). Control values are b) 28.6±0.6 ng/10 min (n=8, glucose-induced insulin release), c) 63.1±2.1 ng/30 min (n=8, arginine-induced insulin release) and d) 39.2±0.6 ng/10 min (n=70, arginine-induced glucagon release), respectively. Data are shown as means ± S.E.M. The number of observations are in parentheses. *P <0.05, **P <0.01, significantly different from each control by the paired b), d) and unpaired * t-test, and t)P<0.01, significantly different from the response to QGPP by the unpaired t-test. g) Not significantly different from the control value (P=0.05).

Fig. 2. Typical data showing the effect of 194 nM salivary peptide P-C (P-C) on arginine (10 mM)-induced immunoreactive insulin (IRI) level. Arginine-stimulated IRI release was measured during a 30 min period, from 0 min to 30 min with (solid lines) and without (dotted lines) simultaneous administration of P-C. The right panel indicates the total IRI level during the 30 min of arginine stimulation in the presence (a hatched column) and in the absence of (an open column) P-C. Data are shown as means ± S.E.M. of 5–8 observations. **P <0.01, significantly different from the response to arginine alone by the unpaired t-test. Note that P-C potentiates the total amount of arginine (10 mM)-induced insulin release.

The salivary glands and the pancreas are closely related in terms of functional similarity (8, 9). The presence of an insulin-like material has been demonstrated in parotid and submandibular glands (10, 11). These findings indicate a close endocrine relationship between the salivary glands and the pancreas. The salivary peptide P-C (P-C), a saliva-derived proline-rich polypeptide, mediates both insulin and glucagon
release. The P-C used in the present study is devoid of crossreactivity to insulin, glucagon, kallikrein or other peptides (12). We have reported that P-C potentiated glucose-induced IRI release and inhibited arginine-induced IRG release in a concentration-dependent manner (4). In order for P-C to potentiate insulin release, the presence of a high concentration of glucose (8.3 mM and higher) seems to be necessary (4).

GLP-1 that exists in the human gut is released into the circulation after a meal (13, 14). GLP-1, at 2–3 times the physiological postprandial plasma concentration, leads to a 3-fold increase in plasma insulin and a brisk fall in plasma glucose from fasting hyperglycemia to the normal (14). GLP-1 has a potent stimulatory effect on insulin release and a slight inhibitory effect on glucagon release (5) in a manner similar to that of P-C, but the sequences comprising GLP-1 are quite different from those of P-C. Histidine (His) at position 7 in GLP-1 is very important for its insulin releasing activity as well as its slight inhibitory effect on glucagon activity (15). In the present study, the P-C-(12–19) fragment was the most potent in inhibiting arginine-induced glucagon release. We suggest that QGPP acts as a structural amino acid sequence and HQ acts as the functional sequence in inhibiting glucagon release. His is also important in the glucagon-inhibiting activity of P-C.

In the present study, P-C significantly potentiated both glucose- and arginine-induced insulin release. The ratio of the potentiating effect of arginine-induced insulin release in the presence of P-C was similar to that in glucose-induced insulin release. The pancreatic B and A cells are equipped with several metabolic pathways to regulate insulin and glucagon release, respectively. The effects of P-C fragments may result from changes in second messenger systems that influence insulin and glucagon release. However, the exact mechanisms involving P-C fragments remain elusive.

In conclusion, two separate functional centers in P-C, fragments P-C-(12–18) and P-C-(23–29), act respectively to potentiate glucose-stimulated IRI release and to inhibit arginine-stimulated IRG release. Both are essential amino acid domains in P-C and contain a common fragment, QGPP. KP and HQ may act as functional amino acid sequences in potentiating insulin and inhibiting glucagon release, respectively.

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