Salivary Peptide P-C Modulates Both Insulin and Glucagon Release from Isolated Perfused Rat Pancreas

Masayasu KIMURA, Noboru NAKASHIMA and Ikuko KIMURA

Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, Toyama 930-01, Japan

Accepted December 27, 1989

Abstract—The effect of salivary peptide P-C, saliva-derived peptide on glucose-induced insulin release was studied using perfused rat pancreas. Salivary peptide P-C (194 nM) remarkably potentiated glucose (8.3 and 16.7 mM)-induced insulin release, whereas the same concentration suppressed arginine (10 mM)-induced glucagon release. Both effects of salivary peptide P-C occurred in a concentration-dependent manner. These findings suggest that salivary peptide P-C may modulate both the levels of insulin and glucagon in vivo.

A new polypeptide, salivary peptide P-C, has been recently isolated from human whole saliva (1, 2). From ontogenic studies on its occurrence, salivary peptide P-C-like immunoreactivity has been shown to appear in fetal B cells after the 16th week of gestation. At the fetal stage before the appearance of salivary peptide P-C, insulin release never occurs, even though insulin biosynthesis has already started in the majority of pancreatic B cells (3). Salivary peptide P-C, however, is a polypeptide having a 44-amino acid sequence that is chemically quite different from insulin and insulin precursors. Antibody against salivary peptide P-C shows no cross-reactivity with insulin, human C peptide, pancreatic polypeptide and other peptide (4). The above-mentioned findings suggest that salivary peptide P-C may be involved in the insulin release from normal pancreatic B cells. The present study was conducted to investigate the effect of salivary peptide P-C on the modulation of insulin and glucagon release from perfused rat pancreas.

Materials and Methods

Male Wistar rats, weighing 200–300 g, were anesthetized with sodium pentobarbital (Nembutal, Abbott Lab.; 50 mg/kg, i.p.); and then the pancreas was isolated along with the stomach, duodenum and spleen in order to keep the pancreas intact, using the methods of Grodsky et al. (5) and Penhos et al. (6). All vessels leaving the pancreas were ligated, except for the portal vein. The superior mesenteric artery was also ligated. Perfusion was carried out through the celiac artery with a pressurized gas mixture consisting of 95% O₂ and 5% CO₂ using a basal medium of Krebs-Ringer bicarbonate buffer (pH 7.4) containing 0.5% bovine serum albumin (Fraction V, Sigma), 2% dextran (T-70, Pharmacia), and 2.8 mM D-glucose according to Niki et al. (7). Salivary peptide P-C (H-GRPQGPPQQGHQQGPPPQGKPQGOPQGRPO 40 44 GPPQGQSPQ-OH) was isolated by Prof. S. Itoh (Dept. of Internal Medicine, Niigata University School of Medicine), and it was also chemically synthesized by Toray Research Institute. Theophylline monohydrate (Nacalai Tesque) and arginine HCl (I-isomer, Wako) were used.

The concentrations of insulin and glucagon collected from a portal vein catheter were determined using the radioimmunoassay previously reported (8). All data were statistically analyzed by the unpaired t-test.

Results

Potentiating effect of salivary peptide P-C on glucose-induced insulin release: Salivary
peptide P-C potentiated glucose (16.7 mM)-induced increase of immunoreactive insulin (IRI) level from perfused rat pancreas. The potentiation was concentration-dependent in the range of 97 to 776 nM of the peptide, for repeated 10-min exposures with the experimental design illustrated in Fig. 1. These concentrations of salivary peptide P-C alone had no effect on the insulin release. The potentiating effect of 194 nM salivary peptide P-C on 16.7 mM glucose-induced IRI release was confirmed to be of the same extent for repeated 7-min exposures using the method shown in Fig. 2. The potentiation by salivary peptide P-C for glucose-induced IRI release was reproducible for 3 stimulation trials.

The insulin-releasing response was saturated at 388–766 nM of the peptide as shown in Table 1, where the measured values of the concentration-response relation are shown. The release ratios were estimated for the value without salivary peptide P-C (16.7 mM glucose alone). The extent of increase was about three times greater than that of 16.7 mM glucose alone. The EC50, with 95% confidence limits, of salivary peptide P-C was 134 (103–173) nM, and the submaximal response was obtained at 388 nM or the peptide, and estimated to be 42% of the maximal response for 8 mM theophylline, a phosphodiesterase inhibitor, with 16.7 mM glucose as a positive control. The IRI level was 7.91 ± 0.44 mU/10 min (316.4±17.6 ng/10 min) (4 observations) at the same concentration of theophylline with 5.6 mM glucose.

The insulin-releasing effects were observed continuously in a time-course from 0 to 30 min after the administration of 194 nM salivary peptide P-C with 8.3 mM glucose (Fig. 3). The peptide potentiated both the first and second phases of glucose-induced increase of IRI level. The total amount of insulin release with the peptide was about two times greater than that without peptide.

The potentiation was induced at higher concentrations of glucose (above 8.3 mM). Peptide at 97–776 nM had no effect on IRI release with a basal medium containing 2.8 mM glucose and 5.6 mM glucose (data not shown). This indicated that the effect of

![Fig. 1](https://example.com/figure1.png)

**Fig. 1.** Potentiating effect of salivary peptide P-C (97–776 nM) on 16.7 mM glucose-induced immunoreactive insulin (IRI) level from perfused rat pancreas. The columns show the period (10 min) of stimulation by 16.7 mM glucose with or without salivary peptide P-C and 8 mM theophylline. Closed circles show IRI level every 1 min. Dotted lines show the ending of stimulation and washing with basal medium. Note that salivary peptide P-C potentiated 16.7 mM glucose-induced insulin release in a concentration-dependent manner.
salivary peptide P-C was dependent on the presence of a stimulative concentration of glucose.

**Inhibitory effect of salivary peptide P-C on arginine-induced glucagon release:** Salivary peptide P-C at 194–776 nM significantly inhibited the amount of immunoreactive glucagon (IRG) released by 10 mM arginine. When determined for repeated 10-min exposures using the experimental method shown in Fig. 4, the inhibitory effect was concentration-dependent. The decrease of IRG level by salivary peptide P-C was not due to the repeated effect of arginine stimulation (data not shown). The measured values of the concentration-inhibition relation for salivary peptide P-C are summarized in Table 2. The release ratios were estimated for the value without the peptide (10 mM arginine alone). The IC50, with 95% confidence limits, of salivary peptide P-C was 194 (161–235) nM. The maximum inhibition by 388 nM of the peptide was estimated to be 58% of the control value without the peptide.
Fig. 3. Typical data for the sequential effect of 194 nM salivary peptide P-C on 8.3 mM glucose-induced IRI level. The glucose stimulation was continuous from 0 min to 30 min after the simultaneous administration of salivary peptide P-C (a solid line and a hatched column). The right panel indicates the total amount of IRI level for the 30 min-stimulation. The broken line and the open column show the effects of 8.3 mM glucose alone. Data are shown as means±S.E.M. of different pancreas. The observed number was 6-7. **P<0.01, significantly different from the effect of glucose alone by Student's t-test. Note that salivary peptide P-C totally potentiated 8.3 mM glucose-induced insulin release.

Fig. 4. Inhibitory effect of salivary peptide P-C (97–776 nM) on 10 mM arginine-induced immunoreactive glucagon (IRG) level. The columns show the period (10 min) of stimulation by 10 mM arginine with or without salivary peptide P-C. Closed circles show IRI level every 1 min. Dotted lines show the ending of stimulation and washing with basal medium. Note that salivary peptide P-C inhibited 10 mM arginine-induced glucagon release in a concentration-dependent manner.

The time course of IRG release was observed continuously from 0 to 30 min after administration of 194 nM salivary peptide P-C (Fig. 5). The total amount of arginine-
Table 2. Inhibitory effect of salivary peptide P-C on 10 mM arginine-induced IRG level from perfused rat pancreas

| Arginine (mM) | P-C (nM) | IRG level (ng/10 min) | Release ratio against that for arginine |
|---------------|----------|-----------------------|----------------------------------------|
| 10            | 0        | 38.0±1.7              | 1.00                                   |
| 10            | 97       | 37.6±2.7              | 0.99                                   |
| 10            | 194      | 28.6±2.4**            | 0.75                                   |
| 10            | 388      | 22.0±2.4**            | 0.58                                   |
| 10            | 776      | 24.0±1.2**            | 0.63                                   |

Values are shown as means±S.E.M. of 5-8 observations. Significantly difference at **P<0.01 by Student's t-test.

Fig. 5. Typical data for the sequential effect of 194 nM salivary peptide P-C on arginine-stimulated IRG level. The arginine stimulation was continued for 30 min, from 0 min to 30 min after the simultaneous administration of salivary peptide P-C (a solid line and a hatched column). The right panel indicates the total amount of IRG level for the 30 min-stimulation. The broken line and the open column show the effect of 10 mM arginine alone. Data are shown as means±S.E.M. of 5-6 observations. **P<0.01, significantly different from the effect of arginine alone by Student's t-test. Note that salivary peptide P-C totally inhibited 10 mM arginine-induced glucagon release.

Induced increase of IRG level was inhibited significantly by 194 nM salivary peptide P-C, and it was 75% of the value with arginine alone.

The effect of salivary peptide P-C on arginine-induced glucagon release was compared with the effect on insulin release. The effects of salivary peptide P-C on both insulin and glucagon release were estimated individually in Tables 1 and 2. A G/I curve was replotted from both sets of data (Fig. 6). The maximum I/G ratio was 8.7 (g/g) at 388 nM of the peptide. The G/I curve illustrates that the effect of salivary peptide P-C depended on both the potentiating effect of insulin release and the inhibitory effect of glucagon release.

Discussion

Since the discovery of a nerve growth factor, a large number of polypeptides have been investigated in attempts to extract a new bioactive factor from salivary glands (9). From submaxillary gland have been isolated epidermal growth factor (10), glucagon (11) or R-mesodermal growth factor (12), the protein fraction of a lipolytic factor (13) and neurite-inducing factor (14). From parotid secretions, on the other hand, a small peptide with glycolytic activity (15), Fr. AA-1 (16), fractionated from parotin (17) and a histidine-
Fig. 6. Concentration-response curves for salivary peptide P-C expressed as a ratio (g/g) to the 10 mM arginine-induced increase of IRG level for the 16.7 mM glucose-induced increase of IRI level. Data are replotted from Tables 1 and 2 with means±S.E. The observed number was n=5-8. Note that the effect of salivary peptide P-C depends totally on the potentiated effect of insulin release and the inhibitory effect of glucagon release.

rich polypeptide (18) have been identified. The salivary peptide P-C used in the present study is also a saliva-derived proline-rich polypeptide, but differs from the other polypeptides in its ability to potentiate the insulin release from isolated perfused rat pancreas.

Insulin release has been reported to be stimulated also by peptide hormones such as secretin (19), gastrin (19), cholecystokinin (pancreozymin) (10), vasoactive intestinal polypeptide (VIP), pancreatic inhibitory polypeptide (GIP) and β-endorphin. There are, however, some significant differences between the patterns of action of these secretagogues, including salivary peptide P-C. VIP (20) and β-endorphin (21) stimulate not only insulin release, but also glucagon release, whereas GIP (22) stimulates only insulin release, which is characterized by potentiation of the effect of glucose. Salivary peptide P-C selectively stimulated B cells and potentiated insulin release induced by glucose. The characteristic feature of salivary peptide P-C was to suppress glucagon release, while at the same dose augmenting insulin release.

In the mechanism by which salivary peptide P-C potentiates insulin release, the presence of a high concentration of glucose (8.3 mM and moreover) seems to be an essential condition. In the pancreas in diabetic (non-insulin-dependent diabetes mellitus) patients, the decrease of positive immunofluorescence due to salivary peptide P-C-like immunoreactivity was frequently demonstrated (23). The decrease in the level of salivary peptide P-C may, therefore, be a significant factor in the occurrence of diabetes. We reported the anti-hyperglycemic effect of salivary peptide P-C on alloxan-induced diabetic mice (24).

In conclusion, the present study provides evidence that salivary peptide P-C plays a possible role in regulating both glucose-induced insulin release and arginine-induced glucagon release from perfused pancreas, thus controlling the maintenance of normoglycemia. This finding may be useful for future studies on the interaction between the saliva-derived peptide and the function of the pancreatic islets.

Acknowledgments: The authors wish to thank Prof. A. Niki (Aichi-Gakuin University) for his helpful advice on perfusion technique, and Prof. S. Itoh (Dept. of Internal Medicine, Niigata University School of Medicine) and the Basic Research Laboratories of Toray Industries, Inc. for kindly supplying us with salivary peptide P-C.

References
1 Isemura, S., Saitoh, E. and Sanada, K.: Isolation and amino acid sequences of proline-rich peptides of human whole saliva. J. Biochem. 86, 79-86 (1979)
2 Isemura, S., Saitoh, E. and Sanada, K.: The amino acid sequence of a salivary proline-rich peptide P-C and its relation to a salivary proline-rich phosphoprotein, protein C. J. Biochem. 87, 1071-1077 (1980)
3 Ito, S., Isemura, S., Saitoh, E., Sanada, K., Suzuki, T. and Shibata, A.: Ontogeny of salivary peptide P-C-like immunoreactivity in the human pancreatic B cells. Acta Endocrinol. 103, 552-557 (1983)
4 Ito, S., Isemura, S., Saitoh, E., Sanada, K., Suzuki, T. and Shibata, A.: Immunohistochemical demonstration of salivary peptide P-C-like immunoreactivity in the human pancreatic B cells. Acta Endocrinol. 103, 544-551 (1983)
5 Grodsky, G.M., Batts, A.A., Bennett, L.L., Vcella, C., McWilliams, N.B. and Smith, D.F.: Effects of carbohydrates on secretion of insulin from isolated rat pancreas. Am. J. Physiol. 205, 638-644 (1963)
6 Penhos, J.C., Wu, C., Basabe, J.C., Lopez, N. and Wolff, F.W.: A rat pancreas-small gut preparation for the study of intestinal factor and insulin release. Diabetes 18, 733-738 (1969)
7 Niki, A., Niki, H. and Miwa, I.: Effect of anomers of D-mannose on insulin release from perfused rat pancreas. Endocrinology 105, 1051-1054 (1979)
8 Kimura, M., Suzuki, J. and Amemiya, K.: A genetically diabetic model "KK-CAY mice" for a pharmacological assay. Endocrinol. Japon. 26, 185-195 (1979)
9 Cohen, S.: Purification of a nerve growth-promoting protein from the mouse salivary gland and its neurocytotoxic antiserum. Proc. Natl. Acad. Sci. U.S.A. 46, 302-311 (1960)
10 Cohen, S.: Isolation of a mouse submaxillary gland protein accelerating incisor eruption and eyelid opening in the newborn animal. J. Biol. Chem. 257, 1555-1562 (1982)
11 Lawrence, A.M., Kirsteins, L., Hojvat, S., Rubin, L. and Poloyan, V.: A potent extrapancreatic hyperglycemic factor. Clin. Res. 23, 536A (1975)
12 Haraguchi, K.H., Knox, R.J., Weimar, V.L. and Anderson, R.A.: Synergistic growth stimulation of corneal fibroblasts by components of mesodermal growth factor from murine submaxillary glands. J. Cell Physiol. 111, 117-132 (1982)
13 Zimowska, U.: Certain characteristic features of the lipolytic factor from rat submaxillary salivary glands. Acta Physiol. Pol. 34, 555-562 (1983)
14 Wagner, J.A.: NIF (neurite-inducing factor): A novel peptide inducing neurite formation in PC12 cells. J. Neurosci. 6, 61-67 (1986)
15 Holbrook, I.B. and Molan, P.C.: The identification of a peptide in human parotid saliva particularly active in enhancing the glycolytic activity of the salivary micro-organisms. Biochem. J. 149, 489-492 (1975)
16 Aonuma, S., Kohama, Y., Komiyama, Y., Fujimoto, S. and Nomura, M.: Amino acid sequence of an active fragment, FrAA-1 of salivary gland hormone. Chem. Pharm. Bull. (Tokyo) 28, 417-423 (1980)
17 Ito, Y.: Parotin: A salivary gland hormone. Ann. N.Y. Acad. Sci. 65, 228-312 (1960)
18 Oppenheim, F.G., Yang, Y.C., Diamond, R.D., Hyslop, D., Offner, G.D. and Troxler, R.F.: The primary structure and functional characterization of the neutral histidine-rich polypeptide from human parotid secretion. J. Biol. Chem. 261, 1177-1182 (1986)
19 Dupre, J., Curtis, J.D., Unger, R.H., Waddell, R.W. and Beck, J.C.: Effects of secretin, pancreozymin, or gastrin on the response of the endocrine pancreas to administration of glucose or arginine in man. J. Clin. Invest. 48, 745-757 (1989)
20 Schebalin, M., Said, S.I. and Maklouf, G.M.: Stimulation of insulin and glucagon secretion by vasoactive intestinal peptide. Am. J. Physiol. 232, E197-E200 (1977)
21 Verdonk, C.A., Rizza, R.A., Nelson, R.L., Go, V.L.W., Gerich, J.E. and Service, F.J.: Interaction of fat-stimulated gastric inhibitory polypeptide on pancreatic alpha and beta cell function. J. Clin. Invest. 65, 1119-1125 (1980)
22 Feldman, M., Kiser, R.S., Unger, R.H. and Li, C.H.: Beta-endorphin and the endocrine pancreas. Studies in healthy and diabetic human beings. N. Engl. J. Med. 308, 349-353 (1983)
23 Ito, S., Suzuki, T., Momotsu, T., Tsuda, A., Takai, M., Shibata, A., Isemura, S., Saitoh, E. and Sanada, K.: A study on salivary peptide P-C-like immunoreactivity in the human pancreas, with special reference to the pathogenesis of NIDDM. Folia Endocrinol. Japon. 59, 1123-1130 (1983)
24 Kimura, M. and Nakashima, N.: Anti-hyperglycemic action of salivary peptide P-C on the diabetic state: Possible mechanisms for regulation of both insulin and glucagon release form isolated perfused rat pancreas. Abst. of 8th Int. Congress of Endocrinol. (Kyoto), p. 490 (1988)