Rapid Publications

Effects of Synthetic Human Pancreatic Polypeptide, Synthetic Bovine Pancreatic Polypeptide, and the C-Terminal Hexapeptide on Pancreatic Somatostatin and Glucagon Secretion in the Rat

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SUMMARY

Synthetic human pancreatic polypeptide stimulated pancreatic somatostatin secretion by isolated rat islets and by the isolated perfused rat pancreas. In contrast, synthetic bovine pancreatic polypeptide and the C-terminal hexapeptide had no effect on somatostatin secretion. Synthetic human pancreatic polypeptide had only a mild stimulatory effect on glucagon secretion at the highest concentration of the peptide used (2.2×10^{-6} M) and in the presence of 16.7 mM glucose. DIABETES *31*:178–181, 1982.

Although the role of pancreatic polypeptide (PP) is not fully established, it is known that at physiologic concentrations, it will inhibit pancreatic exocrine secretion in man[1] and the dog.[2] In mammals, it is found principally in the islets of Langerhans and the exocrine pancreas.[3] In addition, material with immunoreactive properties similar to avian PP has been identified in central and peripheral neurons.[4,5] Since some PP cells in the pancreas have long cytoplasmic processes that abut on both neighboring endocrine cells and exocrine cells, it seems possible that PP may exert some direct control on the function of other endocrine cells as well as on the exocrine pancreas.[6] An apparent preferential association between the cytoplasmic processes of the PP cells and the pancreatic D cell may have been due to the relative proximity of PP and D cells.[6]

Previous reports have given variable results on the effects of PP on both insulin[7–10] and somatostatin[11,12] secretion. The different results could have been due to differences of species, tissue, or PP preparation. Many preparations of natural PP contain sufficient somatostatin[13] and glucagon (unpublished observation) to affect pancreatic endocrine secretion independently of any specific PP effect. Therefore, synthetic human PP (hPP) and synthetic bovine PP (bPP) were used in this series of experiments to investigate the effect of PP on pancreatic somatostatin and glucagon secretion. In addition, the effect of the synthetic C-terminal hexapeptide was investigated since the C-terminal region of the molecule common to hPP, bPP, and other mammalian PPs,[14] is essential for the biologic activity of PP on the exocrine pancreas.[15,16]

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MATERIALS AND METHODS

Male Wistar rats weighing 180–250 g were used throughout. Rats were killed between 0830 and 0900 h in the fed state, and pancreatic islets were isolated by a modification of the collagenase technique.[17] Islets were preincubated for 30 min at 37°C in a modified Krebs-Ringer bicarbonate buffer (KRB-Hepes) pH 7.4 and subsequently incubated for 30 min in groups of 10 islets in 1 ml of KRB-Hepes buffer containing the test substances, as previously described in detail.[18]

Perfused pancreas. Rats used as pancreas donors were taken in the fed state and were anesthetized with thiopental, 100 mg/kg i.p. The procedure used to isolate and perfuse the pancreas was that described by Penhos et al.[19] with minor modifications, previously described in detail.[20] In brief, the pancreas was perfused at a rate of 4 ml/min with modified Krebs-Ringer bicarbonate buffer that contained 4% Dextran, 0.2% human serum albumin, and 400 KIU/ml aprotinin. The basal perfusate contained, in addition, 2.8 mM glucose. The equilibration period, during which the pancreas was perfused with basal perfusate, was 30 min (−30 to 0 min). The somatostatin[18] and glucagon[21] assays used to measure hormone secretion by islets and the perfused pancreas have been described previously.

Source of materials. Aprotinin (Trasylol): Prof. G. L. Haberland, Bayer AG, Wuppertal, F.R.G. Synthetic hPP: Dr. C. A. Meyers, Tulane University School of Medicine, New Orleans, Louisiana. Synthetic bPP: Bachem, Bubendorf, Switzerland. C-terminal hexpeptide of hPP and bPP: U.C.B., Brussels, Belgium.

Presentation of results. The various PP preparations were

TABLE 1
Effect of synthetic hPP, synthetic bPP and the C-terminal hexapeptide on somatostatin secretion by isolated islets in the presence of 2.8 mM and 16.7 mM glucose, mean ± SEM

Glucose		Peptide	Somatostatin secretion* at the various peptide concentrations				
			2.2×10^{-11} M	2.2×10^{-9} M	2.2×10^{-7} M	1.1×10^{-6} M	2.2×10^{-6} M
2.8 mM	+	synthetic hPP	121† ± 6.9 (18)	118† ± 6.6 (19)	496‡ ± 19.6 (19)	2760‡ ± 70.7 (7)	5740‡ ± 183 (7)
2.8 mM	+	synthetic bPP	—	—	—	116 ± 21 (7)	122 ± 23.6 (7)
2.8 mM	+	hexapeptide	—	120 ± 16 (16)	150 ± 21 (16)	—	—
16.7 mM	+	synthetic hPP	101 ± 4.7 (18)	165 ± 35.1 (16)	314‡ ± 17.5 (17)	1750‡ ± 44.2 (6)	3560‡ ± 118 (7)
16.7 mM	+	synthetic bPP	—	—	—	111 ± 16.4 (7)	138 ± 11.6 (7)
16.7 mM	+	hexapeptide	—	78.6 ± 10.6 (16)	98.6 ± 11.6 (16)	—	—

* Somatostatin secretion in the presence of the various peptides is expressed as a percent of control for the individual experiments. Control, somatostatin secretion in absence of PP, for all experiments was: (a) 2.8 mM glucose—24 ± 2pg/10 islets/30 min (45), and (b) 16.7 mM glucose—41 ± 3pg/10 islets/30 min (46) [(a) vs. (b), $P < 0.001$].
† $P < 0.05$ vs. control.
‡ $P < 0.001$ vs. control.

studied in separate experiments. For purposes of interexperimental comparison, the effects of hPP, bPP, and the hexpapetide on hormone secretion have been expressed as a percentage of control values in each experiment (Tables 1 and 2).

Statistical analyses were carried out using Student's unpaired *t* test.

RESULTS

Somatostatin secretion. Synthetic hPP caused a marked stimulation of somatostatin secretion while synthetic bPP and the C-terminal hexapeptide had no significant effect (Table 1). In the presence of 2.8 mM glucose, synthetic hPP caused significant stimulations of somatostatin secretion at all concentrations tested (range 2.2×10^{-11} M to 2.2×10^{-6} M). Glucose alone (i.e., 16.7 mM vs. 2.8 mM) caused a significant increase in somatostatin secretion (41 ± 3 vs. 24 ± 2 pg/10 islets/30 min, $P < 0.001$). At 16.7 mM glucose, the stimulatory effect of synthetic hPP on somatostatin secretion was seen at concentrations of 2.2×10^{-7} M and above (Table 1). The amount of somatostatin secreted at 2.8 mM glucose in the presence of 1.1×10^{-6} M and 2.2×10^{-6} M synthetic hPP was 680 ± 18 (N = 7) and 1420 ± 45 (N = 7) pg/10 islets/30 min, respectively. During perfusion of the isolated rat pancreas (N = 1) the basal somatostatin secretion rate in the presence of 2.8 mM glucose was 120 pg/min. Two minutes after the addition of 1.1×10^{-6} M synthetic hPP the secretion rate was increased approximately 40-fold and at 3 min, the secretion rate was increased 60-fold. The effects of synthetic hPP on somatostatin secretion were immediately reversible (Figure 1). Synthetic bPP, tested at the same concentration in a separate experiment (N = 1), had no effect on somatostatin secretion from the isolated perfused rat pancreas (results not shown).

Glucagon secretion. Synthetic hPP had little effect on glucagon secretion. At 16.7 mM glucose and at the highest concentration of the peptide tested, 2.2×10^{-6} M, a stimulatory response was noted (Table 2).

DISCUSSION

Our results have shown that synthetic hPP is a potent stimulator of pancreatic somatostatin secretion and the pancre-

TABLE 2
Effect of synthetic hPP on glucagon secretion by isolated islets in the presence of 2.8 mM ang 16.7 mM glucose, mean ± SEM

	Glucagon secretion* at the various peptide concentrations				
	2.2×10^{-11} M	2.2×10^{-9} M	2.2×10^{-7} M	1.1×10^{-6} M	2.2×10^{-6} M
2.8 mM glucose + synthetic hPP	117 ± 30.5 (17)	76.7 ± 13.7 (19)	137 ± 24.6 (19)	128 ± 18.7 (7)	102 ± 6.1 (7)
16.7 mM glucose ± synthetic hPP	115 ± 19.2 (19)	124 ± 21.2 (18)	129 ± 18.8 (17)	125 ± 9.6 (6)	177† ± 18.1 (7)

* Glucagon secretion in the presence of synthetic hPP is expressed as a percent of control for the individual experiments.
Control, glucagon secretion in absence of PP, for all experiments was: (a) 2.8 mM glucose—298 ± 45 pg/10 islets/30 min (23) and (b) 16.7 mM glucose—272 ± 28 pg/10 islets/30 min (24).
† $P < 0.05$ vs. control.

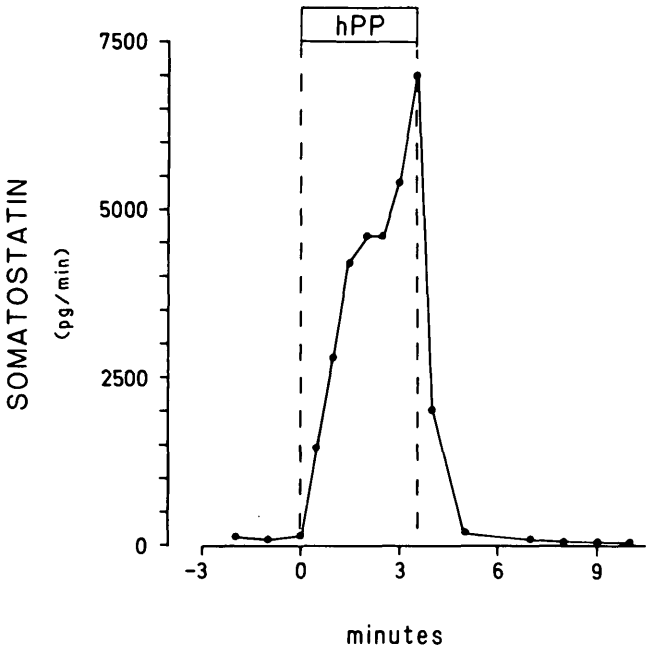

FIGURE 1. Effect of synthetic hPP (1.1×10^{-6} M) in the presence of 2.8 mM glucose on somatostatin secretion by the isolated perfused rat pancreas. Synthetic hPP (1.1×10^{-6} M) was infused between 0 and 3.5 min.

atic D cells responded to synthetic hPP in a dose-dependent manner. The pancreas perfusion experiment showed that the D-cell secretory response to synthetic hPP was immediate and that on withdrawal of the peptide the secretion rate immediately returned to the prestimulatory level. The same concentration of hPP elicited a similar fold increase in somatostatin secretion from the islets as from the perfused pancreas. At the highest dose of hPP used, approximately 30–40% of islet somatostatin content was secreted in 30 min (somatostatin contents not shown), which is higher than the maximum obtained by combined stimulation with glucose and cyclic AMP but similar to the percent content of insulin secreted in the latter condition.[20] By contrast, synthetic bPP had no effect on pancreatic somatostatin secretion. Since hPP and bPP carry the same C-terminal hexapeptide, which is considered essential for the biological effects of PP on the exocrine pancreas,[15,16] the failure of bPP and the C-terminal hexapeptide to affect pancreatic somatostatin secretion suggests that there may be a second biologically active site on the hPP molecule outside the C-terminal region.

Purified natural bPP has also been shown to have no effect on pancreatic D-cell secretion in the dog.[22] In contrast to the lack of effect of bPP on pancreatic somatostatin secretion in the rat, an inhibitory effect of bPP on gastric somatostatin secretion has been reported[12] showing that in the same species the localization of the D cell may modulate its response to PP. Synthetic hPP (prepared by the same group as the hPP used in the present experiments) caused a *fall* in portal and peripheral plasma somatostatin levels when injected intravenously in the rat.[11] As it is presently believed that gastric and intestinal somatostatin secretions are the principal contributors to portal and peripheral plasma somatostatin concentrations,[23,24] the fall in these concentrations with i.v. injection of synthetic hPP can, along with the results of the present experiments, be taken as evidence of opposite effects of hPP on intestinal and pancreatic somatostatin secretion. However, it is also possible that the fall in plasma somatostatin levels reported after i.v. injection of hPP may have been due to indirect rather than direct effects of the peptide on the D cell.

In conclusion, synthetic hPP is a potent stimulator of pancreatic somatostatin secretion in the rat. The absence of similar effects by synthetic bPP suggests the presence of a second biologically active site on the hPP molecule outside the C-terminal hexapeptide region.

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REFERENCES

[1] Greenberg, G. R., McCloy, R. F., Adrian, T. E., Chadwick, V. S., Baron, J. H., and Bloom, S. R.: Inhibition of pancreas and gallbladder by pancreatic polypeptide. Lancet *2*:1280–82, 1978.

[2] Taylor, I. L., Solomon, T. E., Walsh, J. H., and Grossman, M. I.: Pancreatic polypeptide. Metabolism and effect on pancreatic secretion in dogs. Gastroenterology *76*:524–28, 1979.

[3] van Noorden, S., and Falkmer, S.: Gut-islet endocrinology—some evolutionary aspects. Invest. Cell Pathol. *3*:21–35, 1980.

[4] Lorén, I., Alumets, J., Håkanson, R., and Sundler, F.: Immunoreactive pancreatic polypeptide (PP) occurs in the central and peripheral nervous system: preliminary immunocytochemical observations. Cell Tissue Res. *200*:179–86, 1979.

[5] Lundberg, J. M., Hökfelt, T., Änggård, A., Kimmel, J., Goldstein, M., and Markey, K.: Coexistence of an avian pancreatic polypeptide (APP) immunoreactive substance and catecholamines in some peripheral and central neurons. Acta Physiol. Scand. *110*:107–109, 1980.

[6] Rahier, J., and Wallon, J.: Long cytoplasmic processes in pancreatic polypeptide cells. Cell Tissue Res. *209*:365–70, 1980.

[7] Adrian, T. E., Bloom, S. R., Hermansen, K., and Iversen, J.: Pancreatic polypeptide, glucagon and insulin secretion from the isolated perfused canine pancreas. Diabetologia *14*:413–17, 1978.

[8] Lundquist, I., Sundler, F., Ahren, B., Alumets, J., and Håkanson, R.: Somatostatin, pancreatic polypeptide, substance P, and neurotensin: cellular distribution and effects on stimulated insulin secretion in the mouse. Endocrinology *104*:832–38, 1979.

[9] Williams, R. H., and Champagne, J.: Effects of cholecystokinin, secretin and pancreatic polypeptide on secretion of gastric inhibitory polypeptide, insulin and glucagon. Life Sci. *25*:947–56, 1979.

[10] Murphy, W. A., Fries, J. L., Meyers, C. A., and Coy, D. H.: Human pancreatic polypeptide inhibits insulin release in the rat. Biochem. Biophys. Res. Commun. *101*:189–93, 1981.

[11] Arimura, A., Meyers, C. A., Case, W. L., Murphy, W. A., and Schally, A. V.: Suppression of somatostatin levels in the hepatic portal and systemic plasma of the rat by synthetic human pancreatic polypeptide. Biochem. Biophys. Res. Commun. *89*:913–18, 1979.

[12] Chiba, T., Tamínato, T., Kadowaki, S., Abe, H., Chihara, K., Matsukura, S., Goto, Y., Seino, Y., and Fujita, T.: Effects of insulin and pancreatic polypeptide on gastric somatostatin release. Diabetes *29*:292–95, 1980.

[13] Samols, E., and Harrison, J.: Remarkable potency of somatostatin as a glucagon suppressant. Metabolism *25*:1495–97, 1976.

[14] Floyd, J. C., Fajans, S. S., Pek, S., and Chance, R. E.: A newly recognized pancreatic polypeptide; plasma levels in health and disease. Recent Prog. Horm. Res. *33*:519–70, 1977.

[15] Lin, F. M., Evans, D. C., Chance, R. E., and Spray, G. F.: Bovine pancreatic peptide: action on gastric and pancreatic secretion in dogs. Am. J. Physiol. *232*:E311–15, 1977.

[16] Konturek, S. J., Meyers, C. A., Obtulowicz, W., Oleksy, G., and Schally, A. V.: Difference in the action of human pancreatic polypeptide (PP) and its C terminal hexapeptide on pancreatic secretion in man and dogs. Reg. Pep. *1* (Suppl. 1):S62 (abstract), 1980.

[17] Lacy, P. E., and Kostianovsky, M.: Method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes *16*:35–39, 1967.

[18] Trimble, E. R., Gerber, P. P. G., and Renold, A. E.: Abnormalities of pancreatic somatostatin secretion corrected by in vivo insulin treatment of streptozotocin-diabetic rats. Diabetes *30*:865–67, 1981.

[19] Penhos, J. C., Wu, C. H., Basabe, J. C., Lopez, N., and Wolff, F. W.: A rat pancreas—small gut preparation for the study of intestinal factor(s) and insulin release. Diabetes *18*:733–38, 1969.

[20] Gerber, P. P. G., Trimble, E. R., Wollheim, C. B., Renold, A. E., and Miller, R. E.: Glucose and cyclic AMP as stimulators of somatostatin and insulin secretion from the isolated perfused rat pancreas: a quantitative study. Diabetes *30*:40–44, 1981.

[21] Trimble, E. R., Halban, P. A., Wollheim, C. B., and Renold, A. E.: Functional differences between rat islets of ventral and dorsal pancreatic origin. J. Clin. Invest. In press.

[22] Ipp, E., Dobbs, R. E., McCorkle, K., Harris, V., and Unger, R. H.: Further evidence for similar secretory responses of pancreatic somatostatin and insulin. Clin. Res. *26*:33A, 1978. Abstract.

[23] Taborsky, G. J.: Contribution of pancreatic secretion and extraction to plasma somatostatin-like immunoreactivity (SLI). Diabetes *30* (Suppl. 1): 1A, 1981. Abstract.

[24] Marre, M., Attali, J. R., Helman, A., Miller, J., Kronheim, S., and Assan, R.: Pancreatic and gastric secretions of somatostatin-like immunoreactivity (SLI) in rat portal vein: an in vivo and in vitro comparative study. Diabetologia *21*:302, 1981. Abstract.