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HUMAN PANCREATIC POLYPEPTIDE INHIBITS INSULIN RELEASE IN THE RAT

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SUMMARY

Synthetic human pancreatic polypeptide (hPP) administered intravenously suppressed hepatic portal insulin levels in a dose-dependent manner in both fed and fasted rats. No effect on glucagon levels was detected. The C-terminal hexapeptide (hPP_{31-36}) and the C-terminal decapeptide (hPP_{27-36}) exhibited no effect on insulin levels in fed rats at molar doses significantly higher than the effective doses of hPP. A physiological role for hPP in the control of insulin is suggested.

INTRODUCTION

Since the initial isolation and characterization of pancreatic polypeptides from several species (1,2), various studies aimed at defining the physiological role of PP have been undertaken (see (3) for review). These studies have now been facilitated by the availability of large quantities of peptide obtained through solid-phase synthetic techniques. This methodology also results in the ready availability of synthetic peptide fragments as well. The majority of the biological studies performed thus far have centered on PP effects on the exocrine pancreas, gall bladder, gastrointestinal motility or gastric secretion (3,4). Given the immunocytochemical localization of the hPP cells predominantly at the periphery of the pancreatic islets (5), a possible paracrine function might be expected. Our group previously reported suppression of plasma somatostatin levels by synthetic hPP (6) and these studies regarding hPP effects on endocrine pancreatic function have now been extended to determine whether synthetic hPP can also alter hepatic portal insulin and glucagon levels in the rat.

Abbreviations: hPP, human pancreatic polypeptide; bPP, bovine pancreatic polypeptide.

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METHODS

Peptide synthesis: The synthesis of hPP_{1-36} was previously reported by our group (7). For the synthesis of hPP_{31-36} and hPP_{27-36} a Beckman 990 automatic synthesizer was used to assemble each peptide on benzhydrylamine resin (Bachem, Torrence, Calif.) using BOC-L-amino acids (Bachem) and 33% trifluoroacetic acid/methylene chloride deprotection.

The hexapeptide, hPP_{31-36} (0.25 mmole) was cleaved from the resin by HF in the presence of 10% anisole and 80 mg dithiothreitol, and purified by gel filtration on Sephadex G-25 with 2N AcOH. Most of the material, 99 mg, was pure at this point. A few of the fractions showed minor impurities by TLC which were removed by partition chromatography. The combined pure peptide, 135 mg, (67% yield) was homogeneous in four TLC systems and gave the correct amino acid analysis for hPP_{31-36}.

The decapeptide, hPP_{27-36} (0.50 mmole) with the terminal BOC group removed, was cleaved from the resin by HF with 15% anisole and purified on Sephadex G-25 with 2N AcOH. The resulting material, 590 mg, (88% yield) was homogeneous in four TLC systems without further purification. The amino acid analysis was correct for hPP_{27-36}.

Animals: Male, Charles River CD strain rats weighing 230-320g were used in all bioassays. Weight difference within a single assay was less than 50g. The animals were maintained under controlled temperature (24 $\pm$ 2°C) and light (0500-1900 h) conditions for at least one week prior to an assay. Diet consisted of Purina rodent laboratory chow and tap water *ad libitum*.

Insulin and glucagon bioassays: Fed rats or rats that were fasted for 27-30 h (to raise plasma glucagon levels) were used as indicated. All assays were conducted from 1230-1500 h. The rats were anesthetized with sodium pentobarbital (Nembutal) (5mg/100g BW, ip). Exactly 21 min following Nembutal injection, 0.5 ml of isotonic saline or saline containing the test peptide was injected into the jugular vein over a 1-min period. Exactly 5 min after beginning the iv injection 4.0 ml of blood was quickly drawn from the hepatic portal vein following rapid laparotomy.

Blood samples: Immediately after withdrawal, blood samples were transferred to chilled test tubes containing EDTA (2.5mg/ml blood) and Trasylol (500 Kallikrein Inactivator Units/ml blood) (FBA Pharmaceuticals, New York). Plasma was separated by centrifrugation. Aliquots of the plasma were distributed into clean test tubes, frozen in acetone/dry ice and stored at -20°C until assayed for hormone concentration.

Plasma hormone determinations: Plasma insulin levels were determined using matched insulin RIA kits (Cambridge Nuclear Radiopharmaceuticals, Billerica, MA). Plasma glucagon was determined by a standard method using crystalline glucagon (Eli Lilly) for standards, rabbit antiserum 30K (Unger) against glucagon and [^{125}I] iodoglucagon (Cambridge Nuclear Radiopharmaceuticals) as tracer.

RESULTS AND DISCUSSION

The data presented in Table I clearly indicate that iv administration of synthetic hPP results in a dose-dependent suppression of hepatic portal insulin levels in both fed and fasted rats. On a molar basis, the doses of hPP re-

Table I. Effect of synthetic hPP on hepatic portal plasma levels of insulin and glucagon in fed and fasted rats.

DOSE (ug hPP/100g BW)	INSULIN (uU/ml)	GLUCAGON (pg/ml)
	FED	
0	120 ± 7 (5)	64 ± 13 (5)
2	75 ± 3 (5)	58 ± 5 (5)
8	55 ± 8 (5)	62 ± 5 (5)
	FASTED	
0	50 ± 7 (4)	120 ± 21 (5)
4	39 ± 4 (4)	115 ± 11 (5)
12	21 ± 4 (4)	107 ± 9 (5)

All values are the mean ± SEM (n).

quired to produce this suppression of insulin (2-12ug/100g BW) are only approximately twice the amount required for somatostatin to produce a similar degree of inhibition in the same bioassay (see, e.g. (8)). This suggests that suppression of insulin is a physiologic rather than a pharmacologic activity of hPP. The somewhat higher doses of hPP required to produce suppression in fasted rats as opposed to fed rats may indicate a possible role of blood glucose in mediating this activity of hPP. Total suppression of insulin was not achieved at doses as high as 30ug/100g BW (data not shown). Other control mechanisms may be involved which prevent lowering of insulin by hPP below some minimal level. It is interesting that hPP had no effect on plasma glucagon levels in either fed or fasted rats (Table I). The significance of the inhibition of insulin but not of glucagon is at present unclear, especially since food is a strong stimulus for PP release (9,10).

The C-terminus of bovine pancreatic polypeptide (bPP) was found to be required for inhibition of gastric acid secretion and pancreatic enzyme secretion in the dog (11). Additionally, the C-terminal hexapeptide of bPP was

TABLE II. Effect of synthetic hPP_{31-36} and hPP_{27-36} on hepatic portal plasma insulin levels in fed rats.

PEPTIDE	DOSE (ug/100g BW)	INSULIN (uU/ml)
hPP_{31-36}	0	99 ± 6
hPP_{31-36}	1	94 ± 7
hPP_{31-36}	4	99 ± 18
hPP_{27-36}	0	98 ± 8
hPP_{27-36}	3	107 ± 10
hPP_{27-36}	9	106 ± 12
hPP_{27-36}	18	99 ± 11

Values are the mean ± SEM; n=5 for all.

found to mimic the actions of bPP_{1-36} on gastric and exocrine pancreatic secretions as well as gut motility, but at molar doses higher than that required by the entire bPP molecule (3,4). In the present study, the C-terminal hPP hexapeptide, identical in sequence to the bPP hexapeptide, produced no effect on insulin levels at a molar dose approximately 12 times greater than that required by hPP_{1-36} to suppress insulin (Table II). Several biosynthetic peptide hormone precursors are characterized by the presence of two consecutive basic residues immediately preceding the actual hormone sequence (8,12,13). Inspection of the amino acid sequences of the several pancreatic polypeptides characterized to date (3) reveals that the basic amino acid arginine is present at residues 25 and 26 in all the species where the sequence is known and, in all, the C-terminal decapeptide sequence is conserved. For this reason, the C-terminal decapeptide, hPP_{27-36}, was synthesized. Again, as with hPP_{31-36}, no effect on insulin levels was observed with the decapeptide at molar doses 30 times greater than that of hPP_{1-36} (Table II). Therefore, it appears that the insulin-release inhibiting activity of PP, unlike exocrine pancreatic activity, does not emanate from the C-terminal region. Further biological testing with N-terminal fragments is presently being performed.

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