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Pancreatic secretory trypsin inhibitor (PSTI) isolated from pig intestine

Influence on insulin and somatostatin release

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Upon investigation of pig intestinal peptides for effects on the release of endocrine hormones from the isolated perfused rat pancreas, we reported earlier that glucose-stimulated insulin release was inhibited by PEC-60, a peptide with marked sequence similarity to PSTI (pancreatic secretory trypsin inhibitor). Continuing this study we found a polypeptide, which inhibited glucose-induced insulin release but enhanced glucose-induced somatostatin secretion. Determination of the amino acid sequence of this polypeptide revealed that it is identical to that of PSTI. Thus, PSTI modulates islet hormone release.

Pancreatic secretory trypsin inhibitor; Pig intestine; Trypsin inhibitor; Perfused rat pancreas

1. INTRODUCTION

Two types of trypsin inhibitors were originally isolated from bovine pancreas; the Kunitz (or basic) type of inhibitor [1] and the Kazal (or acidic) type [2]. The latter is secreted into the pancreatic juice and therefore known as pancreatic secretory trypsin inhibitor (PSTI). It has an amino acid sequence of 56 residues with three disulfide bridges [3]. PSTI has been isolated from the pancreas of several species (cf. [3]) as well as from human stomach and rat liver [4,5]. We previously showed that the diazepam binding inhibitor (DBI) from pig intestine inhibited glucose-mediated insulin release from the isolated perfused rat pancreas [6]. A similar effect on insulin release was demonstrated with a novel porcine intestinal polypeptide designated as PEC-60 (peptide with N-terminal glutamic acid (E), C-terminal cysteine (C), and a total of 60 residues). PEC-60 exhibited a marked sequence similarity to PSTI, but did not inhibit trypsin [7]. We extended these experiments by testing effects on insulin release of further fractions from the purification of PEC-60. A peptide, which inhibited glucose-induced insulin release and stimulated glucose-induced somatostatin release was found. This polypeptide was identical to porcine PSTI and inhibited trypsin.

2. MATERIALS AND METHODS

2.1. *Materials*

Carboxymethyl cellulose CM-22 was from Whatman (Kent, UK), Sephadex G25 fine and coarse from Pharmacia (Uppsala, Sweden), L-(1-tosylamido-2-phenyl)ethyl chloromethyl ketone (TPCK)-treated trypsin from Worthington (Freehold, NJ), S-2222 (*N*-benzoyl-Ile-Glu-Gly-Arg-*p*-nitroanilide HCl and its Glu γ-methylester) from Kabi (Stockholm, Sweden) and BAEE (*N*-benzoyl-L-arginine ethyl ester) from Sigma (St. Louis, MO).

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2.2. *Starting material*

A concentrate of thermostable intestinal peptides (CTIP) from pig upper intestine was prepared [8], dissolved in water and fractionated with isopropanol to produce fraction F1 [6]. F1 was chromatographed on Sephadex G25 fine in 0.2 M acetic acid resulting in four fractions, the last of which was saturated with NaCl to produce a precipitate, subsequently collected by suction filtration. This fraction (CTIP-F1-Sx4) was the starting material for further purification, as described in section 3.

2.3. *Perfusion of isolated rat pancreas*

Male Sprague-Dawley rats weighing 200–250 g, fed ad libitum on a standard pelleted diet, were used to study effects of peptides on pancreatic hormonal secretion. After anesthetization (i.p. pentobarbital; 100 mg/kg) the pancreatic glands were dissected free from adjacent tissues and placed in a perfusion chamber [9] with Krebs-Ringer bicarbonate solution supplemented with bovine albumin (20 g/l) and glucose (3.3 mM). The medium, infused into the celiac artery, was fed into the isolated pancreas by an open, non-recycling perfusion system with a flow of 2.8 ml/min. The pancreas was equilibrated with the basal medium for 40 min before introduction of 16.7 mM glucose at time zero. The peptide (10 nM) was added to the perfusion medium 10 min before time zero and kept with 16.7 mM glucose during the perfusion period. Samples of the perfusate were collected for radioimmunoassay of insulin [10] and somatostatin [11] in prechilled tubes, containing Trasylol (1000 U).

2.4. *Structural analysis*

Total compositions were determined with an LKB 4151 alpha plus amino acid analyzer after hydrolysis in evacuated tubes with 6 M HCl/0.5% phenol at 110°C for 20 h. Half-cystine was determined as cysteic acid after oxidation with performic acid.

3. RESULTS AND DISCUSSION

Peptide fraction CTIP-F1-Sx4 (cf. section 2.2) (15 g) from pig intestinal preparations was extracted with 750

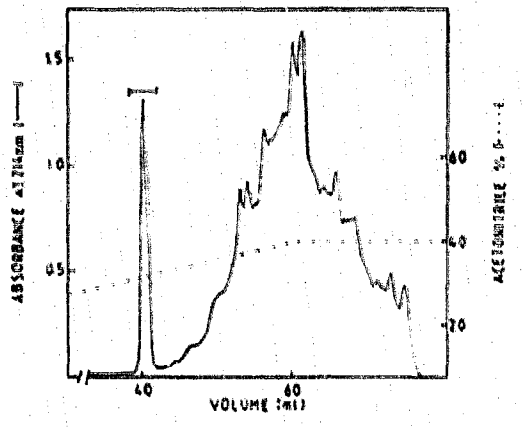

Fig. 1. Nonadsorbed peptides from CM-cellulose chromatography of the methanol-soluble portion of fraction CTIP-F1-Sx4 fractionated on a TSK ODS-120T (10 μm; 7.8×300 mm) in 0.1% trifluoroacetic acid with a gradient of acetonitrile in 0.1% trifluoroacetic acid as shown. Elution of PSTI is indicated with a bar.

Table I

Total composition of porcine intestinal PSTI

Residue	mol/mol
Cys	5.3 (6)
Asx	4.5 (4)
Thr	5.3 (6)
Ser	5.3 (6)
Glx	7.8 (7)
Pro	5.5 (5)
Gly	4.5 (4)
Ala	1.1 (1)
Val	4.1 (4)
Met	0 (0)
Ile	2.8 (3)
Leu	2.5 (2)
Tyr	2.1 (2)
Phe	0 (0)
Lys	4.4 (4)
His	0.2 (0)
Arg	2.1 (2)

Values shown are molar ratios from acid hydrolysis, and, within parentheses from the sum of the sequence analysis. Cys was determined as Cya

ml methanol, the suspension was filtered, brought to pH 7.5±0.1 with 0.1 M NaOH in methanol, and the precipitate formed was removed by filtration. The filtrate was brought to pH 2.7±0.1 with 0.1 M HCl in methanol, after which peptides and dissolved NaCl were precipitated with ether (3 vols). After evaporation of the ether, the precipitate was redissolved in water and peptides were reprecipitated with NaCl. The precipitate (266 mg) was collected by suction filtration. NaCl was removed by chromatography on Sephadex G25 coarse in 0.2 M acetic acid, and the peptide fraction (CTIP-F1-Sx4-MeOH-soluble) was lyophilized (139 mg). The peptide fraction, redissolved in water (10 ml), was further purified on CM-cellulose (1.5×16 cm) in 0.01 M ammonium bicarbonate and adjusted with 0.02 ammonia to pH 8.0±0.1. Nonadsorbed peptides were lyophilized (46 mg) and aliquots (1.5 mg) were chromatographed by reverse-phase HPLC in a Waters system with dual wavelength detection at 215 and 280 nm, a TSK ODS-120T column (10 μm; 7.8×300 mm) and a gradient of 15–40% acetonitrile in 0.1% trifluoroacetic acid at a flow of 1.5 ml/min (Fig. 1). The average yield was 0.3 mg. Essentially pure peptide giving only one component on thin-layer silica gel chromatography in butanol/pyridine/acetic acid/water (15:10:3:12, by volume) and only one N-terminus.

The amino acid sequence of the polypeptide was determined in a Milligen Prosequenser 6600 using DITC coupling. Results were interpretable up to cycle 54. Cycles 9, 16, 24, 35 and 38 showed no identifiable residue. The amino acid sequence obtained was in complete agreement with that of porcine pancreatic PSTI assuming the 'empty' cycles to constitute positions with disulfide-linked half-cystines (3 intrachain disulfide-bridges in PSTI). PSTI has two additional residues, proline and cystine at positions 55 and 56, and the proline was also clearly ascertainable in the total composition (Table I) of the intestinal PSTI.

Our conclusion is that the porcine intestinal PSTI has the same primary structure as porcine pancreatic PSTI. This porcine intestinal PSTI has also been assayed using TPCK-trypsin with S-2222 and BAEE as substrates [12,13], demonstrating a trypsin inhibition at close to equimolar inhibitor amounts, as for pancreatic PSTI [4]. In the isolated, perfused rat pancreas, 16.7 mM glucose induced biphasic responses of insulin and somatostatin (Fig. 2). When PSTI was added to the perfusion medium 10 min prior to and simultaneously with the high glucose concentration, both the early and late phase insulin responses were significantly suppressed (37% and 29%, respectively). Glucose-induced somatostatin response, on the other hand, was stimulated by 25% in the presence of PSTI (Fig. 2, Table II).

PSTI is considered to be produced in acinar cells of the pancreas and is a normal component of the exocrine secretion [14]. Its physiological role has been thought to involve protection of pancreatic cells from autodigestion by preventing trypsin activation of the zymogen [15]. However, in the small intestine the Paneth cells, which morphologically resemble pancreatic acinar cells, contain trypsin and PSTI immunoreactivity [16], as confirmed by immunohistochemical investigations [17]. Immunoreactive PSTI has also been detected in serum [18], particularly from patients after major surgery, injuries or inflammation [19,20], and recently in normal and malignant tissues [21]. In the present study, we have isolated PSTI from porcine intestine, identified its primary structure as identical to that for porcine pancreatic PSTI and

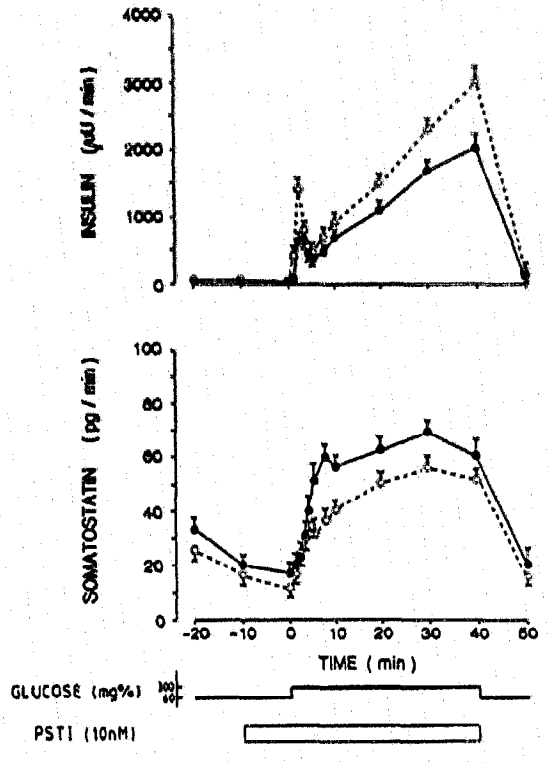

Fig. 2. Effect of PSTI (●) on glucose-induced responses of insulin and somatostatin from the isolated, perfused rat pancreas. Glucose perfusion in the absence (○; $n = 8$) or presence (●; $n = 8$) of 10 nM PSTI from time −10 to +40 min. Insulin and somatostatin in the perfusate measured by radioimmunoassays, values given as mean ± SE.

established a trypsin inhibitory effect. As the intestines were thoroughly emptied and thereafter separately rinsed under pressure with cold water before the isolation procedure, the possible contamination by pancreatic juice was excluded. Thus, we confirm the existence of PSTI in the small intestine, probably derived from Paneth cells [16,17].

The suppressive effect of PSTI on insulin release was similar to that recently described with an equimolar concentration of PEC-60 in the perfused rat pancreas [7]. In contrast to PSTI, however, PEC-60 did not affect somatostatin secretion. Since pancreatic somatostatin may have the potential to inhibitit insulin secretion by paracrine interaction between islet D- and B-cells [22], the question arises whether PSTI suppresses insulin secretion indirectly via its effect on the somatostatin response. Such a paracrine interaction seems less plausible, however, as the inhibition of insulin response was observed earlier than the augmentation of the somatostatin response. Thus, PSTI appears to exert direct effects both on islet B- and D-cell secretion. It is possible that the peptide, if secreted to the circulation, participates in the enteroinsular modulation of islet hormone release.

Table II

Effects of PSTI on glucose-induced insulin and somatostatin responses in the isolated, perfused rat pancreas

Hormone response	Hormone secretion		
	Early phase (0–5 min)	Late phase (5–40 min)	Total (0–40 min)
Insulin (μU)			
Control	3340 ± 330	60 890 ± 2820	64 230 ± 3000
PSTI (10 nM)	2120 ± 170**	43 760 ± 4170**	45 880 ± 4270**
Somatostatin (pg)			
Control	63 ± 12	1275 ± 83	1338 ± 89
PSTI (10 nM)	64 ± 11	1598 ± 106*	1662 ± 107*

Values given as mean ± SE ($n = 8$ perfusions).
*$P < 0.05$, **$P < 0.01$ vs control

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