Volume 263, number 2, 279–280 FEBS 08349 April 1990

The importance of the C-terminal amide structure of rat pancreastatin to inhibit pancreatic exocrine secretion

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Received 19 February 1990

A C-terminal fragment of rat pancreastatin, a 26 residue peptide amide and a fragment without a C-terminal amide were synthesized by Fmoc-based solid phase methods and their biological activities were compared. The rat C-terminal fragment inhibited pancreatic exocrine secretions produced by the intravenous injection of 2-deoxy-D-glucose (a central vagal nerve stimulation), whereas the fragment without a C-terminal amide showed no effect on pancreas. These results indicate that the C-terminal amide of this peptide is necessary to reveal its biological activity.

Pancreastatin; C-terminal amide; (Rat; Pancreas)

1. INTRODUCTION

Pancreastatin, first isolated from porcine pancreas by Tatemoto et al., in 1986, is a 49 amino acid residue peptide with a C-terminal amide structure [1]. Pancreastatin has been shown to inhibit endocrine and exocrine pancreatic secretions [2,3]. We recently found that rat pancreastatin inhibited pancreatic exocrine secretions produced by central vagal nerve stimulation [4], and suggested that pancreastatin acted by means of inhibiting vagal efferent nerve excitation. To examine the biological importance of the C-terminal amide structure of pancreastatin, we compared the effects of a synthetic rat pancreastatin C-terminal fragment (26–51) with or without a C-terminal amide structure on pancreatic exocrine secretions in conscious rats.

2. MATERIALS AND METHODS

A rat pancreastatin C-terminal fragment (26–51) with or without a C-terminal amide was synthesized using Fmoc-DMBH-resin and Fmoc-glycine-*O*-HMP-resin, respectively [5,6]. 2-Deoxy-D-glucose (2DG) was purchased from Sigma, St. Louis, MO.

2.1. *Animal preparations*

Male Wistar rats (314–330 g) were prepared with cannulae draining bile and pancreatic juice (BPJ) separately and with a duodenal cannula to return BPJ to the intestine. The operative procedures used have been described previously in detail [7]. After the operation, rats were placed in modified Bollman-type restraint cages. Experiments were conducted on the fourth postoperative day after 5 h fasting in conscious rats.

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2.2. *Experimental protocols*

BPJ was collected separately at 30-min intervals and the volume of pancreatic juice was determined with a Hamilton syringe. Twenty microliters of pancreatic juice was taken for protein assay, and the rest of the pancreatic juice was mixed with bile and infused into the duodenum by a syringe pump (Harvard Apparatus Compact Infusion Pump, Harvard Apparatus, Southnatick, MA) during the next 30 min. After the 90-min basal collection, 75 or 15 mg/kg of 2DG was injected as a bolus. The infusion of pancreastatin was started at a rate of 1 ml/h 60 min prior to the injection of 2DG.

2.3. *Assays*

The protein concentration in pancreatic juice was determined by optical density at 280 nm of samples diluted 200 times in 0.04 M Tris buffer, pH 7.8 and its output per 30 min was estimated.

2.4. *Analysis of data*

Values were expressed as the mean ± SE. Results were analyzed by multiple analysis of variance (MANOVA) with repeated measures with respect to the treatment and time, followed by Duncan's multiple range test, or one-way analysis of variance (ANOVA) followed by Newman-Keul's multiple comparison test, where appropriate. A value of $P < 0.05$ was considered significant.

3. RESULTS

3.1. *Stimulatory effect of 2-deoxy-D-glucose on rat pancreas*

The injection of 75 mg/kg of 2DG significantly increased pancreatic juice flow and protein output (Fig. 1). The smaller dose of 2DG (15 mg/kg) produced smaller but significant increases in fluid and protein outputs (Table I).

3.2. *Effect of rat pancreastatin with a C-terminal amide on 2DG-stimulated pancreatic secretions*

The continuous infusion of pancreastatin with a C-terminal amide (100 pmol/kg per h) inhibited the pan-

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

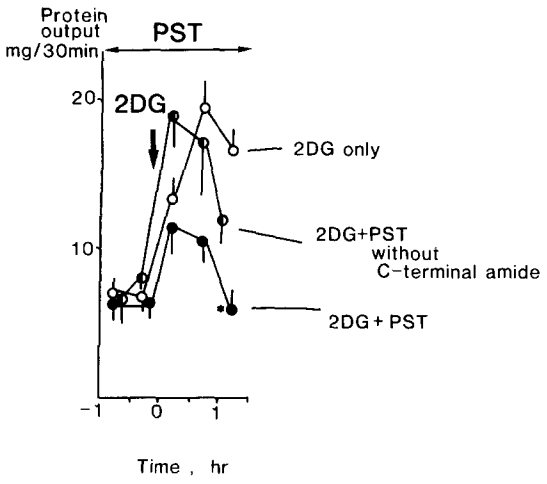

Fig. 1. Changes in protein secretions produced by 2DG injection (75 mg/kg) with 100 pmol/kg per h of pancreastatin (PST) with a C-terminal amide and with 1000 pmol/kg per h of pancreastatin without a C-terminal amide. The F value obtained by repeated measures MANOVA for three treatments was $F_{2,18} = 6.29$, $P < 0.01$. The number of animals is shown in Table I.

creatic responses to both doses of 2DG stimulation (Fig. 1 and Table I). Responses to the smaller dose of 2DG were completely abolished by pancreastatin (Table I).

3.3. *Effect of rat pancreastatin without a C-terminal amide on 2DG-stimulated pancreatic exocrine secretions*

In contrast, rat pancreastatin without a C-terminal amide at a dose of 100 pmol/kg per h did not inhibit pancreatic secretions stimulated by 75 mg/kg of 2DG (Table I). Moreover, we examined the effect of a 10 times higher dose of this peptide on pancreatic secretions stimulated by 75 and 15 mg/kg of 2DG. No effect on the pancreatic secretion was observed (Fig. 1 and Table I).

Table I

Increments of protein outputs stimulated by 2DG with and without rat pancreastatin fragments

Doses of 2DG	Protein output (mg/1.5 h)	
	75 mg/kg	15 mg/kg
Without pancreastatin	28.9 ± 2.1 (n = 9)	11.1 ± 2.1 (n = 5)
Pancreastatin with C-terminal amide (100 pmol/kg per h)	11.6 ± 3.0* (n = 6)	0.8 ± 0.9* (n = 5)
Pancreastatin without C-terminal amide (100 pmol/kg per h)	22.8 ± 2.1 (n = 7)	not tested
Pancreastatin without C-terminal amide (1000 pmol/kg per h)	30.3 ± 6.4 (n = 6)	6.2 ± 3.1 (n = 6)

Values were calculated as follows: the sum of values during 1.5 h after 2DG injection minus the sum of values of 1 h basal secretion (before the injection of 2DG) × 1.5, and expressed as mean ± SE. F values by ANOVA were 4.95, $P < 0.01$ for 75 mg/kg per h and 4.51 for 15 mg/kg per h. * Significantly lower than other values by Newman-Keul's multiple comparison test

4. DISCUSSION

The mechanism of 2DG stimulation of the vagal efferent nerve is believed to be the competition of 2DG with glucose for cell membrane transfer resulting in intracellular glucopenia. Pancreastatin inhibits this stimulation. The present results indicate that in order to elicit inhibitory bioactivity on pancreatic exocrine secretion, a C-terminal carboxyl-amidated structure of pancreastatin is necessary. In a recent study, Greeley et al. [8] reported that chromogranin A, which is considered to be a precursor of pancreastatin [9], inhibited insulin release from isolated rat pancreas although a 10-fold higher concentration of chromogranin A than pancreastatin was needed. Chromogranin A is an acidic glycoprotein of about 50 kDa [9] lacking a C-terminal amide structure. In the present study, we could not observe any inhibitory effect when a 10 times higher dose of pancreastatin without a C-terminal amide was applied, whereas 100 pmol/kg per h of pancreastatin with a C-terminal amide could elicit an inhibitory effect on the pancreatic exocrine secretion. Therefore, we propose that pancreastatin acquires its inhibitory bioactivity on the exocrine pancreas by proteolytic processing of chromogranin A and amidation of the carboxy-terminal of pancreastatin [10].

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