Effects of Pancreastatin on Insulin and Pancreatic Polypeptide Secretion in the Dog

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Abstract

Porcine pancreastatin (1.19 nmol) was administered into the peripheral vein (i.v.) or the third cerebral ventricle (i.t.v.) of dogs and its effect on the secretion of insulin and pancreatic polypeptide (PP) studied. Neither means of administration had any effect on basal and glucose-induced insulin or PP secretion. However, i.v. pancreastatin did inhibit the i.v. CCK-8-induced insulin but not PP release. Pancreastatin may thus play a role in the regulation of insulin secretion in the canine pancreas.

Recently Tatemoto et al. (1986) reported the sequence and biological activity of a putative hormone, pancreastatin, from porcine pancreas. Pancreastatin is a 49-residue peptide, having a glycine amide at its C-terminus. It inhibits glucose-stimulated insulin release from isolated perfused rat pancreas and from isolated rat islets (Tatemoto et al., 1986; Efendic et al., 1987; Silvestre et al., 1988) and may be important in the regulation of insulin secretion.

Pancreastatin is found in the brain, particularly the pituitary gland (Schmidt et al., 1988; Ravazzola et al., 1988). This, together with the wide distribution of chromogranin A, a precursor of pancreastatin (Eiden, 1987; Konecki et al., 1987; Huttner et al., 1987; Iacangelo et al., 1988a, b; Helman et al., 1988) in the brain, indicates that pancreastatin may act on the endocrine pancreas via a neural pathway. The purpose of this study was to examine the effect of pancreastatin administered into the peripheral vein (i.v.) or the third cerebral ventricle (i.t.v.) on insulin and pancreatic polypeptide (PP) secretion in the dog.

Materials and Methods

Animal preparation

Seven mongrel dogs, approximately 12 kg in weight, were used. A twenty-gauge stainless
steel cannula was permanently implanted with dental cement, under sodium thiamylal anesthesia, toward the third cerebral ventricle. The methods used have been described elsewhere (Baba et al., 1983). The experiments were initiated 2 weeks after the operation. The dogs were fasted for 16 hr before the experiments with free access to tap water. During each experiment, they were placed in jackets and allowed to remain in a fully conscious, relaxed state.

**Experimental design**

Intravenous butterfly needles were placed in veins in the forelimbs of the dogs, one for infusion of drugs and one for collection of blood samples. Porcine pancreastatin was prepared as described (Tatemoto et al., 1986). Synthetic cholecystokinin octapeptide (CCK-8) was kindly donated by Prof. N. Yanaihara (Shizuoka University, Shizuoka, Japan). Pancreastatin was dissolved in saline and injected i.t.v. (100 µl) or i.v. for 5 min in a single dose of 1.19 nmol. The 1.19 nmol dosage was chosen since many peptides affect visceral and behavioral functions of dogs at this level, including CCK-8 (Morioka et al., 1986; Sakatani et al., 1987; Inui et al., 1988), corticotropin-releasing factor (Inoue et al., 1989), neuropeptide Y (Morioka et al., 1986; Inoue et al., 1989; Inui et al., 1989), and pancreatic polypeptide (Baba et al., 1983; Inoue et al., 1989).

**Experiment 1:** Six dogs received an i.v. or i.t.v. injection of pancreastatin or saline vehicle as a control. Blood samples were obtained during the basal period and between 5 and 120 min after the injection.

**Experiment 2:** Pancreastatin or saline vehicle was i.v. or i.t.v. injected into 5 dogs, followed by an i.v. bolus injection of glucose (0.5 g/kg body wt). Blood samples were obtained during the basal period and at 0 (just after peptide or saline injection), 2.5, 5, 10, 15, 20, 30, 45, and 60 min after the injection of the glucose.

**Experiment 3:** Seven dogs received an i.v. injection of saline first, then pancreastatin 90 min later. Immediately after the peptide or saline injection, CCK-8 was i.v. injected at a dose of 0.8 nmol for 5 min. This dose of CCK-8 was found to cause a significant increase in plasma insulin and PP concentrations via specific CCK receptors (Inui et al., 1988) of the same magnitude as those seen after ingestion of a meal by dogs (Inui et al., 1983). The first and second administration of CCK-8 did not make any difference in stimulating insulin and PP secretion (Inui et al., 1988). Blood was drawn during the basal period and at 0 (just after saline or pancreastatin injection) 5, 10, 15, 20 and 30 min after the start of CCK-8 administration.

**Laboratory analysis**

Blood samples (1.8 ml) were collected in test tubes containing 400 KIU of aprotinin (Trasylol®, Bayer, Japan) and 1.2 mg EDTA in a total volume of 0.2 ml. After centrifugation, plasma aliquots were rapidly frozen for subsequent PP and insulin assays. Blood glucose was assayed by the glucose oxidase method. Plasma PP was measured with a double-antibody radioimmunoassay as previously reported (Mizuno et al., 1979; Inui et al., 1985). Plasma insulin was measured by a homologous porcine insulin assay using the double antibody separation method.

**Statistical analysis**

Results are expressed as means ± standard error. The integrated PP response (IPPR), integrated insulin response (IIR), and integrated glucose response (IGR) were calculated as the summation over basal levels of the respective hormone or of glucose during the experiment. Data were evaluated by analysis of variance, followed by Dunnett's test. A value of p < 0.05 was considered as a significant difference.

**Results**

Pancreastatin, whether administered i.v. or i.t.v., had no significant effect on plasma insulin, PP and glucose concentrations (not shown).

Glucose administration resulted in a peak plasma glucose concentration of $238.2 ± 31.6$ mg/dl at 5 min and $243.4 ± 25.0$ mg/dl at 2.5 min with i.v. or i.t.v. saline injection, respectively (Figs. 1 and 2). There was a concomitant increase in plasma insulin concentrations from $7.1 ± 0.8$ to $39.2 ± 6.2$ µU/ml and from $8.8 ± 2.2$ to $44.8 ± 6.9$ µU/ml. Pretreatment with i.v. or i.t.v. pancreastatin did not significantly alter the plasma insulin
i.v. Pancreastatin or Saline

i.v. Glucose

Fig. 1. Effect of i.v. pancreastatin on insulin and glucose responses to i.v. injection of glucose.

i.t.v. Pancreastatin or Saline

i.v. Glucose

Fig. 2. Effect of i.t.v. pancreastatin on insulin and glucose responses to i.v. injection of glucose.

Discussion

The present study demonstrates that porcine pancreastatin, a novel brain-gut peptide, modulates insulin secretion in the dog. Pancreastatin inhibited CCK-stimulated insulin release without affecting PP release. However, pancreastatin failed to inhibit basal or glucose-stimulated insulin release whether administered into the vein or into the third cerebral ventricle. Pancreastatin infused via the superior pancreaticoduodenal artery also produced no
Fig. 3. Effect of i.v. pancreastatin on insulin and PP responses to i.v. CCK-8.

Fig. 4. The integrated insulin and PP responses to i.v. CCK-8. In this figure, open or shaded columns indicate saline- or pancreastatin-pretreated groups, respectively.

Inhibition of basal insulin output from the duodenal lobe of the pancreas in pentobarbital-anesthetized dogs (Dunning et al., 1988). Therefore, these results suggest that the effect of pancreastatin on insulin secretion may be limited to modulation of the acute response to some, but not all, insulin secretagogues in the dog.

Only limited information is available about the effect of pancreastatin on endocrine pancreas under in vivo conditions. A recent study demonstrated that pancreastatin injected intravenously suppressed both baseline and glucose- and carbachol-stimulated increase in plasma insulin concentrations in conscious mice (Ahren et al., 1988). Pancreastatin also stimulated basal glucagon secretion and induced a transient hyperglycemia. It was reported that the biological activity of porcine pancreastatin resides in the C-terminal part of the molecule (Tatemoto et al., 1986) which is relatively conserved across the species. However, the structure of porcine pancreastatin has 59–71% homologies to the structures of human, bovine, and rat pancreastatin (Iacangelo et al., 1988b), suggesting considerable species variations which may influence the biological activities.
Recently, we found that i.t.v. pancreastatin (1.19 nmol) does not stimulate ACTH and cortisol secretion. However, i.t.v. pancrastatin tended to reverse the i.t.v. CCK-8-induced satiety effect (unpublished data), suggesting that the effect of pancreastatin may not be limited to the endocrine pancreas. Thus, further studies are required to evaluate the role of pancreastatin on pancreatic islet functions and other visceral and behavioral functions.

Acknowledgements

We are very grateful to Dr. R. E. Chance, Lilly Research Laboratories, Eli Lilly & Co., Indianapolis, IN, USA, for donating highly purified pancreatic polypeptides and antiserum. This work was supported, in part, by Grant-in-Aid for Special Project Research 62770852 from the Ministry of Education, Science and Culture of Japan.

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