Pancreastatin Inhibits Insulin Secretion and Stimulates Glucagon Secretion in Mice

BO AHRÉN, STEFAN LINDSKOG, KAZUHIKO TATEMOTO, AND SUAD EFENDIĆ

Recently a new peptide, pancreastatin, was isolated from porcine pancreatic extracts. It contains 49 amino acids and shows a structural similarity to chromogranin A, which occurs in secretory granules of the endocrine pancreas. Furthermore, pancreastatin has been found to inhibit glucose-induced insulin secretion in the perfused rat pancreas. However, its effects under in vivo conditions have never been studied. We have therefore investigated the effects of this peptide on insulin and glucagon secretion in vivo in the mouse. We found that an intravenous injection of pancreastatin (4.0 nmol/kg) lowered basal plasma insulin concentration at 6 min from 55 ± 8 μU/ml in control mice to 21 ± 7 μU/ml ($P < .01$). The peptide also inhibited the plasma insulin response to both glucose ($P < .01$) and the cholinergic agonist carbachol ($P < .001$). Furthermore, 2 min after injection of pancreastatin, plasma glucagon concentration had increased to 301 ± 19 pg/ml compared to 190 ± 12 pg/ml in control mice ($P < .001$). The peptide did not, however, affect the carbachol-induced plasma glucagon response. In addition, pancreastatin induced a transient hyperglycemia. Combined adrenergic blockade by means of a pretreatment of phentolamine and propranolol did not prevent pancreastatin from exerting its effects on plasma insulin levels, whereas the increase in plasma glucagon levels was abolished. Thus, in the mouse, the newly discovered intrapancreatic peptide pancreastatin *1*) lowers baseline plasma insulin levels, *2*) inhibits glucose- and cholinergically induced insulin secretion, *3*) stimulates baseline glucagon secretion, and *4*) induces hyperglycemia. The effects seen were modest in potency, and the effects on plasma insulin levels were seen also after combined α- and β-adrenoreceptor antagonism. We suggest that pancreastatin could be a regulator of islet function. *Diabetes* 37:281–85, 1988

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Received for publication 23 March 1987 and accepted in revised form 27 August 1987.

During the last two decades, evidence has accumulated showing that the pancreas contains a series of novel peptides with potential influences on islet hormone secretion. Islet peptides may thereby influence islet function by different routes. Peptides released from islet endocrine cells may affect nearby cells by paracrine or endocrine mechanisms. For example, somatostatin and glucagon have been shown to affect the secretion of each other in a paracrine manner (1,2). Alternatively, peptides occurring in intraislet nerves, e.g., galanin, may affect islet hormone secretion by a neurocrine action (3).

Recently, a new peptide, pancreastatin, was isolated from porcine pancreatic tissue and shown to contain 49 amino acids (4). The peptide shows a remarkable structural similarity to chromogranin A (5,6), which occurs in secretory granules of various hormone-producing cells, e.g., the endocrine pancreas (7), and whose function is unknown. Pancreastatin was recently found to significantly inhibit glucose- and arginine-stimulated insulin secretion from the isolated perfused rat pancreas (4,8). Somatostatin secretion was slightly suppressed, whereas glucagon release, if anything, was stimulated by pancreastatin (8). The peptide also inhibited glucose-stimulated insulin release from isolated pancreatic islets (8). The effects of pancreastatin under in vivo conditions have never been studied. Therefore, we performed an in vivo study in the mouse.

MATERIALS AND METHODS

Animals. Female mice of the NMRI strain (Anticimex, Stockholm), weighing 25–35 g, were used. The animals were kept on a standard pellet diet (Astra-Ewos, Södertälje, Sweden)

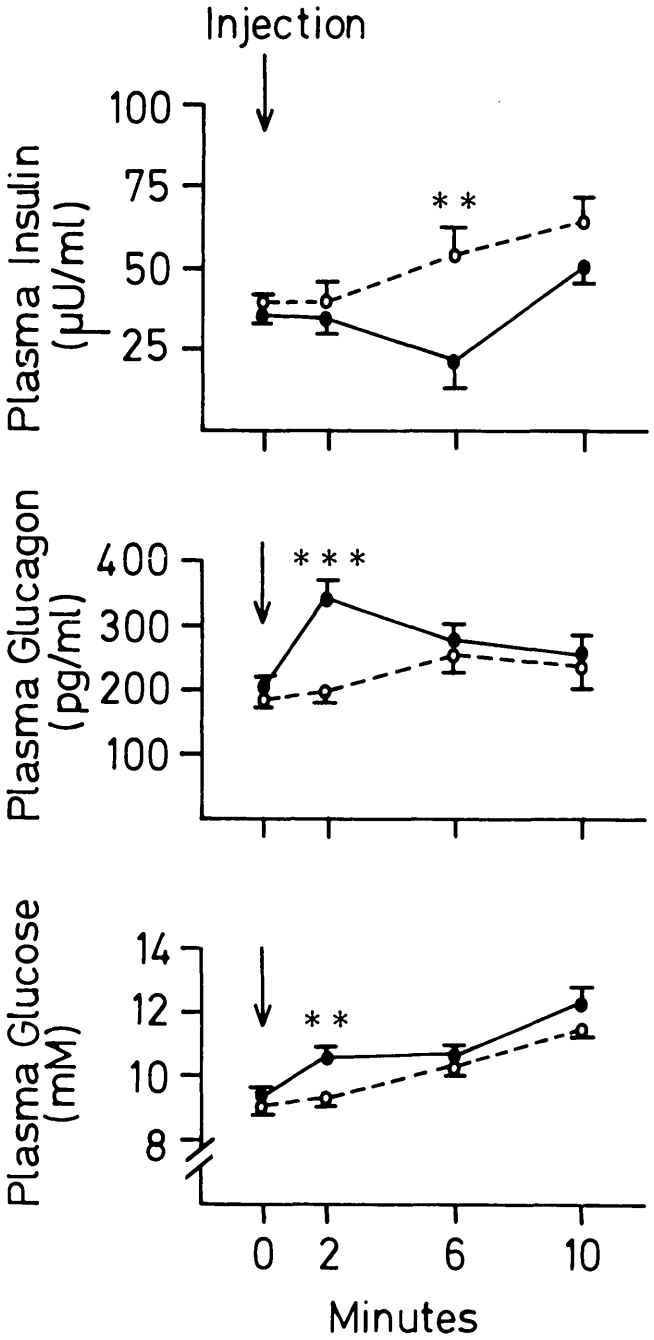

FIG. 1. Plasma levels of insulin (*top*), glucagon (*middle*), and glucose (*bottom*) before and at various time points after intravenous injection of pancreastatin (4.0 nmol/kg; ●, *n* = 20) or saline gelatin (○, *n* = 20). Means ± SE are shown. ***P* < .01, ****P* < .001, probability level of random difference between groups.

and tap water ad libitum before and throughout the experiments. The experiments were performed on unanesthetized animals.

Experiments. Pancreastatin was synthesized as previously described (4); carbachol and D-glucose were obtained from British Drug Houses (Poole, UK). Phentolamine methanesulfonate was from CIBA-Geigy (Basel), and L-propranolol hydrochloride was from ICI (Macclesfield, UK). The substances were dissolved in 0.9% NaCl-0.1% gelatin. In the first experimental series, pancreastatin was injected intravenously into a tail vein (volume load 10 μl/g body wt) at various doses (range 0.5–4.0 nmol/kg body wt). Blood samples were taken by the orbital puncture technique either at 2 min after the injection or as serial sampling immediately before and at 2, 6, and 10 min after the injection. In the second series, carbachol (dose range 16–160 nmol/kg body wt) or D-glucose (dose range 0.6–2.8 mmol/kg body wt) was injected intravenously alone or together with pancreastatin (4.0 nmol/kg body wt), and blood samples were taken at 2 min after the injection. The 2-min time point was chosen because at 2 min after an intravenous injection of carbachol or glucose, plasma insulin and glucagon levels are maximal (9,10). In the third series, 2.6 μmol/kg phentolamine methanesulfonate and 1.6 μmol/kg L-propranolol hydrochloride were injected together intraperitoneally. The doses were selected from previous studies in mice (11,12). Ten minutes later, 4.0 nmol/kg i.v. pancreastatin was injected alone or together with 2.8 mmol/kg D-glucose. Blood samples were taken 2 or 6 min after the intravenous injection. All control animals were injected with saline gelatin. After the sampling, blood was immediately centrifuged and plasma separated and stored at −20°C for later analysis.

Plasma insulin and glucagon were measured by radioim-

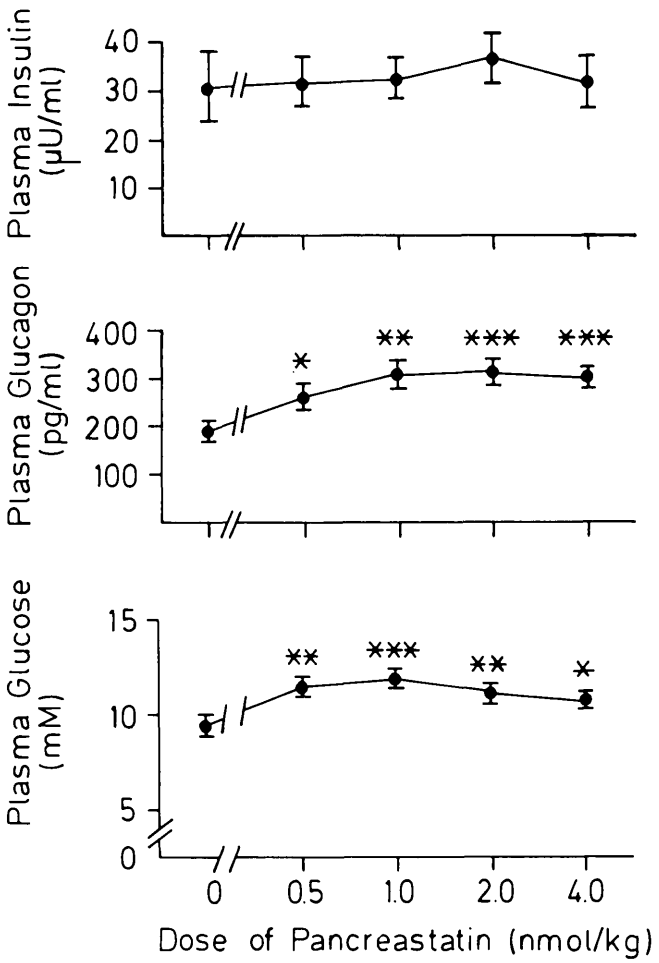

FIG. 2. Relationships between dose of pancreastatin and plasma levels of insulin (*top*), glucagon (*middle*), and glucose (*bottom*). Blood samples were taken at 2 min after intravenous injection. Control mice were given saline gelatin. *n* = 10 in each group. Means ± SE are shown. **P* < .05, ***P* < .01, ****P* < .001, probability level of random difference between experimental and control groups.

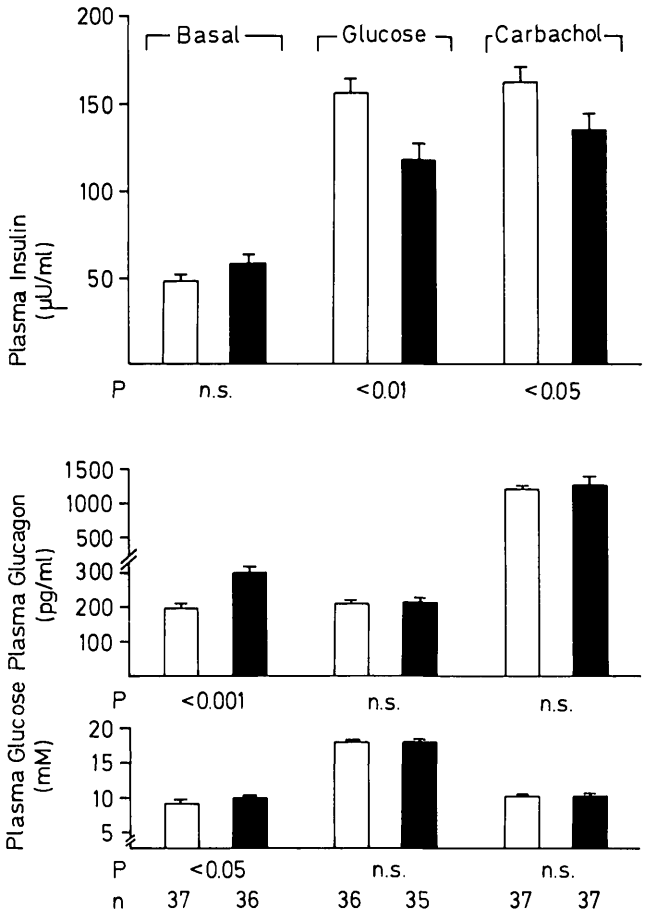

FIG. 3. Plasma levels of insulin (*top*), glucagon (*middle*), and glucose (*bottom*) at 2 min after injection of saline gelatin (basal), 2.8 mmol/kg glucose, or 160 nmol/kg carbachol, alone (*open bars*) or together with 4.0 nmol/kg pancreastatin (*shaded bars*). Means ± SE are shown. *P* = probability level of random difference between groups. *n* = number of animals.

munoassay (13–15), and plasma glucose was determined by a glucose oxidase method (16). Means ± SE are presented. Student's *t* test was used for tests of significance between different groups.

RESULTS

Effects of pancreastatin on baseline plasma levels of insulin, glucagon, and glucose. Figure 1 shows that baseline plasma insulin levels were lowered by a single intravenous injection of pancreastatin (4.0 nmol/kg). The decrease was observed at 6 min after the injection, when plasma insulin concentration was 21 ± 7 μU/ml in animals injected with the peptide, compared to 55 ± 8 μU/ml in control mice ($P < .01$). In contrast, baseline plasma glucagon levels were enhanced by pancreastatin, with the peak at 2 min after injection (Fig. 1). Thus, plasma glucagon at 2 min after injection of pancreastatin was 346 ± 23 pg/ml, compared to 195 ± 15 pg/ml in control mice ($P < .001$). Plasma glucose levels significantly increased at 2 min after the injection of pancreastatin but did not differ from those in control mice at 6 and 10 min after the injection (Fig. 1). Thus, at 2 min after the injection, plasma glucose levels were 9.4 ± 0.2 mM in control mice and 10.6 ± 0.3 mM in animals injected with pancreastatin ($P < .01$).

Figure 2 shows the relationship between the dose of pancreastatin and plasma concentrations of insulin, glucagon, and glucose at 2 min after the intravenous injection. Concentrations of insulin were not different from those in control mice, whereas glucagon concentrations were significantly elevated. This increase was induced by pancreastatin at as low a dose as 0.5 nmol/kg (264 ± 23 vs. 192 ± 18 pg/ml in control mice, $P < .05$). All doses of pancreastatin increased plasma glucose levels.

The decrease in plasma insulin levels at 6 min after pancreastatin injection was repeated by injection of 1.0 nmol/kg pancreastatin (31 ± 6 vs. 59 ± 8 μU/ml, $P < .05$, $n = 10$), whereas at 0.5 nmol/kg, pancreastatin had no influence on basal plasma insulin levels.

Effects of pancreastatin on plasma insulin, glucagon, and glucose after glucose or carbachol injection. Figure 3 shows that the glucose- and carbachol-induced increase in plasma insulin was suppressed by pancreastatin. Thus, pancreastatin decreased the glucose-induced increase in plasma insulin levels from 156 ± 8 μU/ml in control mice to 117 ± 10 μU/ml ($P < .01$). Similarly, pancreastatin suppressed the carbachol-induced increase in plasma insulin levels from 161 ± 9 μU/ml in control mice to 134 ± 9 μU/ml ($P < .05$). By subtracting the basal plasma insulin levels, it is calculated that pancreastatin inhibited glucose- and carbachol-induced insulin responses by 44 and 32%, respectively.

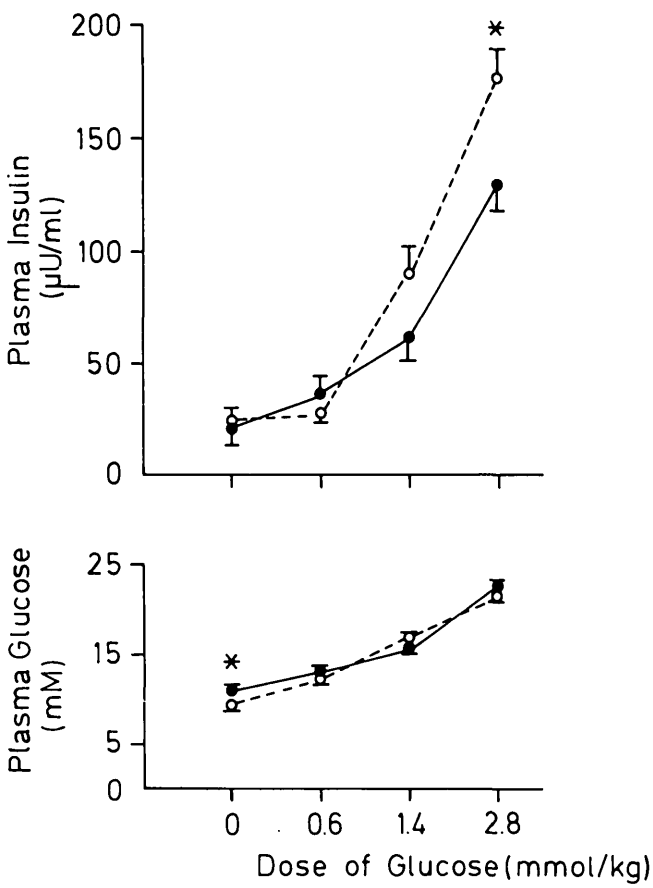

FIG. 4. Plasma levels of insulin (*top*) and glucose (*bottom*) at 2 min after injection of saline gelatin (basal) or various dose levels of glucose, alone (○) or together with 4.0 nmol/kg pancreastatin (●). *n* = 10 in each group. Means ± SE are shown. ***P* < .01, ****P* < .001, probability level of random difference between groups.

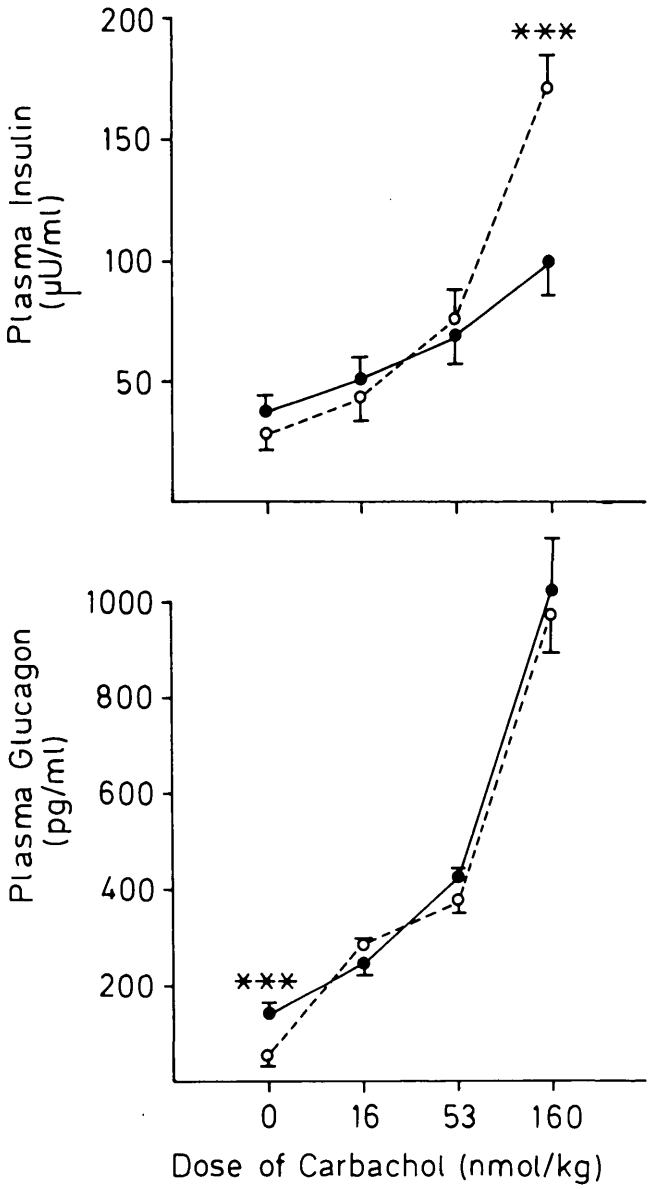

FIG. 5. Plasma levels of insulin (*top*) and glucagon (*bottom*) at 2 min after injection of saline gelatin (basal) or various dose levels of carbachol, alone (○) or together with 4.0 nmol/kg pancreastatin (●). *n* = 18–22 animals in each group. Means ± SE are shown. ****P* < .001, probability level of random difference between groups.

Pancreastatin increased baseline plasma glucagon levels to 294 ± 17 pg/ml compared to 195 ± 14 pg/ml in control mice ($P < .001$; Fig. 3). Glucose abolished this pancreastatin-induced increase in plasma glucagon. The carbachol-induced elevation of plasma glucagon levels was not modulated by pancreastatin.

Figure 4 shows the influence of pancreastatin on plasma levels of insulin and glucose at 2 min after injection of glucose at different dose levels. Pancreastatin suppressed glucose-induced insulin release ($P < .05$). The basal plasma insulin levels were not altered by pancreastatin at 2 min after its injection.

Figure 5 shows the influence of pancreastatin on plasma levels of insulin and glucagon at 2 min after injection of carbachol at different dose levels. Pancreastatin suppressed carbachol-induced insulin release ($P < .001$) but did not affect carbachol-induced glucagon release. The basal plasma glucagon levels were again enhanced by pancreastatin.

Influence of α- and β-adrenoreceptor blockade on the effects of pancreastatin on plasma levels of insulin, glucagon, and glucose. Figure 6 shows that the combined injection of phentolamine and propranolol did not affect plasma insulin levels in the basal state or after injection of glucose but reduced basal plasma glucagon and glucose levels ($P < .01$). Also, pancreastatin after the combined injection of phentolamine and propranolol inhibited glucose-stimulated plasma insulin levels. In contrast, the increase of basal plasma glucagon levels after pancreastatin injection was not seen after pretreatment with phentolamine and propranolol. The lowering of basal plasma insulin levels by pancreastatin of 6 min was unaffected by pretreatment with phentolamine and propranolol: Δ = −28 ± 4 μU/ml without and −26 ± 2 μU/ml with combined α- and β-adrenoreceptor blockade, NS.

DISCUSSION

Pancreastatin was recently isolated from porcine pancreatic extracts by a chemical method that detects the COOH-terminal amide structure in biologically active peptides (17).

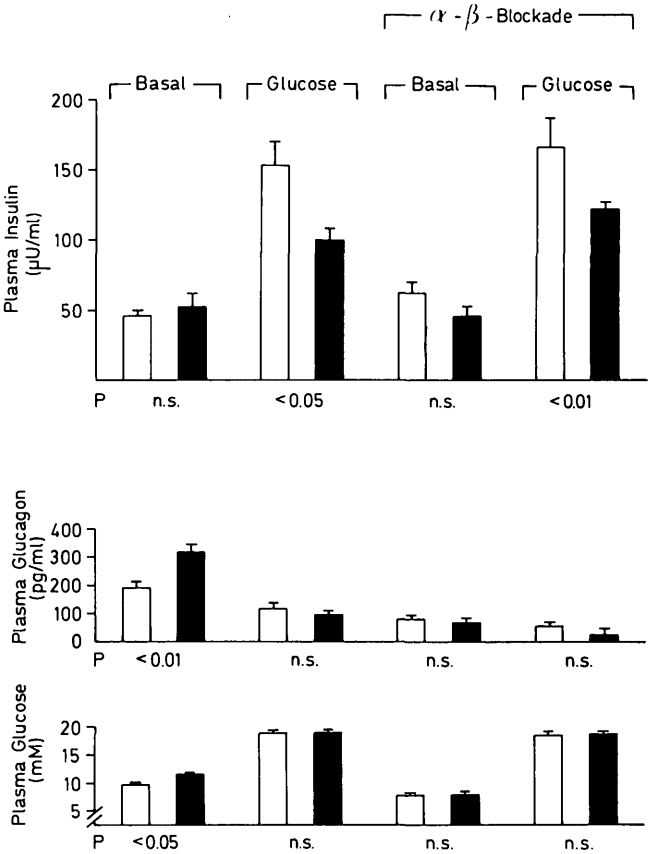

FIG. 6. Plasma levels of insulin (*top*), glucagon (*middle*), and glucose (*bottom*) at 2 min after intravenous injection of saline gelatin (basal) or glucose (2.8 mmol/kg), alone (*open bars*) or together with 4.0 nmol/kg pancreastatin (*shaded bars*). Animals were pretreated with saline gelatin (controls) or a combined intraperitoneal injection of 2.6 μmol/kg phentolamine and 9.6 μmol/kg L-propranolol 10 min before intravenous injection. *n* = 10 animals in each group. Means ± SE are shown. *P* = probability level of random difference between groups.

The peptide was found to contain 49 amino acids with an NH_2-terminal glycine and a COOH-terminal glycine amide structure (4). The same chemical method has earlier been used to isolate a series of biologically active peptides from intestinal and brain extracts. These peptides include neuropeptide Y , PHI 27, peptide YY, galanin, and neuropeptide K (18). The cellular distribution of pancreastatin is not yet established. Recently, however, a great structural similarity between pancreastatin and chromogranin A was observed (5,6). Chromogranin A occurs in secretory granules in peptide-producing cells and in the endocrine pancreas (7) and is released concomitantly with the peptides during secretion. The function of chromogranin A is not yet known, but its great structural similarity to pancreastatin suggests it has a function similar to that of pancreastatin.

Our study is the first investigation on the action of pancreastatin in vivo. We performed our experiments in conscious mice. This could raise concern on possible indirect actions, e.g., increases in circulating catecholamine levels. Because we compared the responses to pancreastatin with those to saline and because combined α- and β-adrenoreceptor blockade could not prevent all actions of pancreastatin, we conclude that the observed differences were due to pancreastatin. We found that pancreastatin suppressed both baseline and glucose- and carbachol-stimulated increases in plasma insulin levels in mice. This agrees with the recent study demonstrating the inhibitory action of pancreastatin on glucose- and arginine-stimulated insulin secretion from perfused rat pancreas and on glucose-stimulated insulin release from isolated rat islets (4,8). The potency of pancreastatin to suppress stimulated insulin secretion was weak, and the peptide did not reach the potency observed with another recently described intrapancreatic peptide, galanin (19).

We also demonstrated that pancreastatin stimulated basal glucagon secretion. In contrast, the peptide did not affect carbachol-induced glucagon secretion, not even when carbachol was injected at submaximal dose levels. Because the increase in plasma glucagon levels was abolished by combined α- and β-adrenoreceptor antagonism, it probably resulted from an indirect action of pancreastatin. As expected, glucose inhibited pancreastatin-induced glucagon secretion in the nonblocked animals. The increase in plasma glucose after pancreastatin injection was anticipated in consideration of the enhanced plasma glucagon levels.

The lowering of the basal plasma insulin levels after pancreastatin injection was seen at 6 min after injection, whereas the elevation of basal plasma glucagon levels was observed at 2 min. The reason behind these different time patterns is an intriguing question that has to await solution. One possibility might be different intracellular events in the α- and β-cells after pancreastatin stimulation; another might be the integrated islet response: a stimulated glucagon secretion might counteract the inhibition of insulin secretion (20). A third possibility is that the fast responses (increase in plasma levels of glucagon and glucose) are indirect, exerted by mechanisms other than through direct peptide-islet interactions. The finding that phentolamine and propranolol prevented these actions leaving those on plasma insulin levels unaffected might support this last assumption.

We therefore conclude that pancreastatin weakly inhibits insulin secretion and stimulates glucagon secretion in vivo. Note that the neuropeptide galanin (19,20) and activation of the pancreatic sympathetic nerves (3) also inhibit insulin and stimulate glucagon release. Because these hormonal alterations characterize non-insulin-dependent diabetes mellitus (21), it is important to study the significance of pancreastatin, galanin, and sympathetic nerves in islet physiology and pathophysiology. The great structural similarity between pancreastatin and chromogranin A (5,6) might also imply that a first function of chromogranin A has been found.

ACKNOWLEDGMENTS

We are grateful to Lena Kvist for technical assistance and to Eva Björkbom for secretarial assistance.

This study was supported by the Swedish Medical Research Council, Stockholm, Sweden (Grants 7X-0034 and 14X-6834); by the National Institute of Medical Health (MH-23862); by Nordisk Insulinfond, Gentofte, Denmark; by the Swedish Diabetes Association, Stockholm, Sweden; and by the Medical Faculty of Lund University, Lund, Sweden.

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