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PANCREASTATIN—A NOVEL REGULATORY PEPTIDE?

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Abstract

Pancreastatin is a 49 amino acid peptide originally isolated from porcine pancreas on the basis of its C-terminal glycinamide as isolation criterion. It is derived by proteolytic processing from chromogranin A, an acidic protein component of secretory granules in endocrine and neuronal cells. The primary structures of human, porcine, bovine and rat pancreastatin have been determined on the protein or cDNA level and show 70% sequence homology. By immunocytochemistry, pancreastatin has been detected in the pituitary, adrenal gland, pancreas, CNS and throughout the gastrointestinal tract. In pancreatic islets, pancreastatin is co-localized with insulin, glucagon and somatostatin. The principle biological activities of this peptide are: inhibition of insulin release and of exocrine pancreatic secretion. These effects which can be assigned to the amidated C-terminal part of the molecule have been demonstrated in several species. Whether or not pancreastatin can be classified as a novel peptide hormone that under physiological conditions plays a role in the regulation of the endocrine and exocrine pancreas, is still a matter of controversy.

Key words: Pancreastatin, gastrointestinal peptide, regulatory peptide, chromogranin, insulin secretion, exocrine pancreatic secretion.

Pancreastatin, a 49 amino acid residues comprising peptide with a C-terminal glycinamide, was isolated from porcine pancreatic extracts by Tatemoto and coworkers (1), using a chemical detection assay for C-terminal amino acid amides as isolation method (2). Using this strategy a number of previously unknown peptide hormones and neuropeptides, i.e. PHI, PYY, NPY and galanin, possessing this characteristic C-terminal structure, have been isolated from brain and intestinal extracts by Tatemoto & Mutt without any knowledge of their respective biological effects or physiological role (3–7). An initial biological screening revealed that natural porcine pancreastatin [1–49] inhibited glucose-induced insulin release from the isolated perfused rat pancreas (1). This activity, which was observed on the first phase and to a lesser degree on the second phase of insulin release, could be assigned to the C-terminal part of the molecule since pancreastatin [14–49] and pancreastatin [33–49], derived by proteolytic cleavage of the parent molecule at single basic residues, showed equal or slightly higher potency (1). The authors implicated by the name 'pancreastatin' that this 49-residue peptide may represent a novel regulatory pancreatic hormone with a physiological role in the control of insulin secretion and carbohydrate metabolism.

Structure and molecular forms

Soon it was recognized that porcine pancreastatin shows striking sequence homology to the central part of the cDNA-derived amino acid sequence of bovine chromogranin A, an acidic protein present in the secretory granules of endocrine and neuronal cells (8–10). It was speculated that chromogranin A may represent the prohormone precursor for pancreastatin (11, 12). This hypothesis was proven by the subsequent characterization of the porcine chromogranin A cDNA structure that contained the full-length sequence of porcine pancreastatin (13). Simultaneously, Konecki et al. (14) and later Helman et al. (15) determined the cDNA structure of human chromogranin A that also contained a pancreastatin-like sequence displaying 70% homology to porcine pancreastatin. The precursor relationship for human chromogranin A/pancreastatin was demonstrated by Schmidt et al. (16) who reported the structural characterization of two human

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pancreastatin-like peptides with C-terminal glycinamides isolated from a carcinoid liver metastasis, comprising 29 and 92 amino acid residues. These peptides were identical in their primary structure to human chromogranin A, position 210–301 and 273–301 respectively. Pancreastatin-92 [210–301] is generated by a trypsin-like cleavage after Lys-Arg [208–209], indicating a putative processing site. The C-terminal part shows 70% sequence homology to porcine pancreastatin and contains the human pancreastatin-like sequence. The second peptide, pancreastatin-29 [273–301] represents a C-terminally amidated fragment of human pancreastatin, generated by cleavage of an acid-labile Asp-Pro bond at the N-terminus, probably during the purification procedure. Surprisingly, a similar C-terminal 28-residue pancreastatin fragment with an identical N-terminus, but missing a glutamic acid residue in the poly-glu region (position 286–290), was isolated from a human glucagonoma (17). Since these authors did not prove their sequence determination by synthesis or mass spectrometry, it cannot be decided whether this structural difference is due to a sequencing error or represents a true polymorphism of the human chromogranin A gene. Two other molecular forms of human pancreastatin-related peptides have been isolated from a human insulinoma metastasis (18): a 186-residue peptide, corresponding to human chromogranin A [116–301], and a 48-residue peptide, identical to human chromogranin A [254–301]. Interestingly, the former peptide, designated pancreastatin-186 [116–301], is the largest pancreastatin-containing derivative of chromogranin A and results from cleavage after a dibasic Lys-Arg residue [114–115] which produces N-terminal chromogranin A peptide betagranin corresponding to human chromogranin A [1–115], originally isolated from rat pancreas by Hutton et al. (19–21). The latter peptide, pancreastatin-48 [254–301], may represent the human equivalent of porcine pancreastatin [1–49], although theoretically other possibilities for the N-terminal processing site still exist, i.e. pancreastatin-53 [249–301] or pancreastatin-52 [250–301]. Desglycinamide-pancreastatin-48 [254–300] was isolated as proteolytic fragment after tryptic digestion, indicating its relative stability despite the existence of a potential tryptic cleavage site at Lys-Gly [276–277] (16). Fig. 1 summarizes in schematic form what has been proposed or proven on the proteolytic processing of human chromogranin A and pancreastatin-related peptides. Since most molecular forms have been isolated from tissue extracts, it still has to be differentiated whether these proteolytic cleavages represent true in-vivo posttranslational processing, in-vivo degradation or in-vitro degradation/cleavage during the isolation and purification procedure. It remains to be shown which molecular form is the quantitatively most abundant species in tissue and, even more important, which form, if any, is found in the circulation or as local modulator under physiological circumstances in different species.

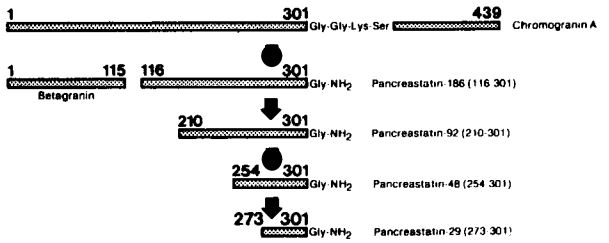

Fig. 1. Schematic overview of the proteolytic processing of human pancreastatin-like peptides derived from chromogranin A. Pancreastatin-29 results from cleavage of an acid-labile Asp-Pro bond (residues 272–273), probably during extraction (16, 17). The numbers represent N-terminal and C-terminal amino acid residues according to the human chromogranin A sequence (14). Modified according to S. Funakoshi et al. (18).

The C-terminal amide assay was subsequently used to isolate pancreastatin from bovine pancreas and pituitary as a 47-residue peptide with C-terminal glycinamide (22), completely identical to the bovine chromogranin A cDNA, thus confirming the precursor relationship independently. Rat chromogranin A cDNA also revealed the existence of a pancreastatin-like sequence, homologous to porcine pancreastatin (23–26). However, rat pancreastatin has not yet been isolated and sequenced on the peptide level. Fig. 2 shows a comparison of the human, bovine, rat and porcine chromogranin A regions containing the pancreastatin-like sequences.

Proteolytic processing of chromogranin A may produce other yet unidentified peptides, which could serve as regulatory peptides (for review see ref. 27). Betagranin has been identified as a 20 kDa protein released from the endocrine pancreas which corresponds to human chromogranin A [1–115] (19–21) whereas a 62 kDa N-terminal fragment has been found in chromaffin granules of the adrenal medulla (28). Osmotically active chromogranin A fragments have been identified after lysis of bovine chromaffin granules (29). Furthermore, bovine chromogranin A can be cleaved by plasma kallikrein, suggesting that processing or degradation of chromogranin A may occur in the circulation (30). Schmidt et al. (16) isolated and partially characterized two peptides derived from the C-terminal part of human chromogranin A. Since a number of dibasic residues are clustered in the C-terminal part of the molecule, it has been claimed that proteolytic processing of chromogranin A starts in this region (8, 28, 31), but definite products of in vivo processing remain to be defined. Plasma kallikrein has been shown to cleave bovine chromogranin A resulting in a long N-terminal and small C-terminal fragments (30).

Tissue distribution, immunocytochemistry, measurement and release of pancreastatin-like peptides

Antisera directed against N- and C-terminal synthetic pancreastatin peptides were raised to characterize tissue

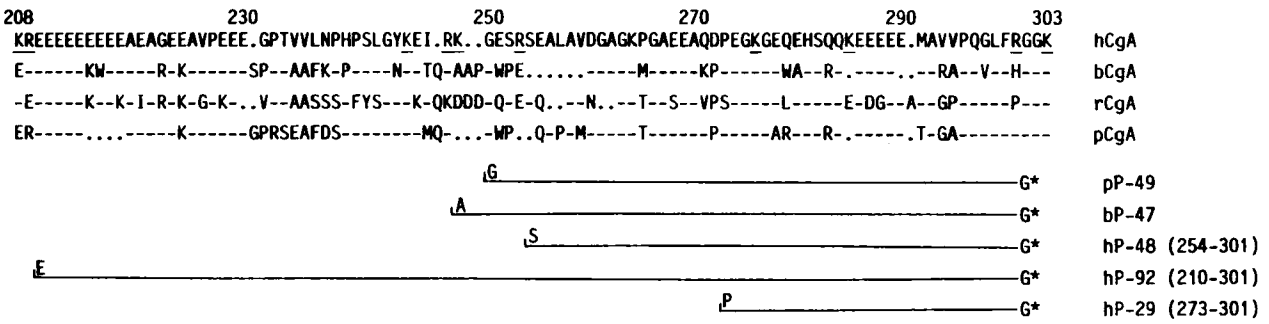

Fig. 2. Comparison of human (hCgA), bovine (bCgA), rat (rCgA) and porcine (pCgA) chromogranin A regions that contain the pancreastatin-like sequences. Amino acid residues are abbreviated according to the single letter code. Residues identical to human CgA are indicated by dashes, dots represent spaces introduced for optimal sequence alignment. Arginine (R) and lysine (K) residues in hCgA are underlined to indicate putative processing sites. pP, porcine pancreastatin; bP, bovine pancreastatin; hP, human pancreastatin; G*, C-terminal glycinamide. Modified according to Schmidt et al. (16).

content and immunocytochemical localization of pancreastatin-like immunoreactivity (PLI). Schmidt et al. (32) demonstrated with a C-terminal specific pancreastatin antiserum that PLI is present throughout the gastrointestinal tract and in most endocrine and neuronal tissues of pig and man. Highest concentrations were measured in the pituitary, adrenal gland and pancreas (1 200–4 000 pmol/g). The tissue distribution was identical to that of chromogranin A and similar, although not identical, to that of chromogranin B (33). Human endocrine tumors contained large quantities of PLI (up to 14 600 pmol/g). HPLC gel permeation chromatography revealed that porcine pancreas and pituitary contained small pancreastatin-like molecules, whereas in adrenal medulla large chromogranin A-like forms predominated. By immunocytochemistry, PLI was localized in pancreatic islets of Langerhans, adrenal medulla, various cell types of the anterior pituitary, ECL- and GI hormone cells of the duodenum and human endocrine tumors (Fig. 3). In islets, insulin, glucagon and somatostatin cells showed intense staining for PLI, whereas PP cells were negative (34). At the electron microscopical level PLI was localized to the electron dense core of the secretory vesicles of the somatostatin and insulin cells and to the less electron dense 'halo' of the glucagon granules. Gastrin, somatostatin and EC cells in the antrum, ECL cells, enteroglucagon and gastrin cells in the duodenum and gonadotrophs and thyrotrophs in the pituitary stained positive for PLI (34). Bretherton-Watt et al. (35) and Ravazzola et al. (36) reported a similar distribution of PLI in porcine gastrointestinal and endocrine tissues. Bishop et al. (37) investigated the expression of PLI in human neuroendocrine tumors. Highest levels were detected in ileal and rectal carcinoids. Kar et al. (38) characterized the distribution of PLI in the central and peripheral nervous system. Highest levels were found in the cortex, thalamus and hypothalamus, immunoreactive fibres were found throughout the brain and spinal cord. PLI was co-localized with CGRP, galanin and other neurotransmitters. In the skin, PLI has been localized in epidermal Merkel cells (39).

The problem of release of PLI into the circulation, being one criterion to be fulfilled for a regulatory hormone, has been addressed by several investigators. Bretherton-Watt et al. (35) reported fasting levels for PLI in unextracted porcine plasma exceeding 2 000 pmol/l. This immunoreactivity consisted mainly of high-molecular weight material, either due to chromogranin A or unspecific interference. After hydrophobic extraction, PLI levels were 90–100 pmol/l, increasing to 130–150 pmol/l in response to a meal. Although it cannot be excluded that this small postprandial rise reflects a systemic regulatory role for pancreastatin, it could perhaps more likely result from an overflow after paracrine release from endocrine cells together with other peptide contents of the secretory granules. Östenson et al. (40) demonstrated that PLI and insulin are released in parellel from the perfused porcine pancreas in response to stimuli which affect insulin release. Released PLI was identified exclusively as a low molecular weight species (5 kDa) (40), thus confirming the tissue processing of pancreastatin in the pancreas (32). Circulating human PLI was detected by Tateishi et al. (41) who found significantly elevated levels in patients with chronic renal failure, liver cirrhosis and neuroendocrine tumors. Extremely high levels of PLI were measured in a patient suffering from a malignant insulinoma with liver metastasis (42). Funakoshi et al. (43) reported significant increases of PLI and CCK in response to the intrajejunal infusion of a liquid mixed meal which was delayed in patients with chronic pancreatitis. The physiological significance of this phenomenon remains to be elucidated.

Although first reports seem to indicate that PLI circulates in plasma in humans and pigs, the mechanism and origin of circulating PLI has to be defined. According to processing studies, the endocrine pancreas may represent a major source for small molecular weight plasma (32, 35, 40, 44).

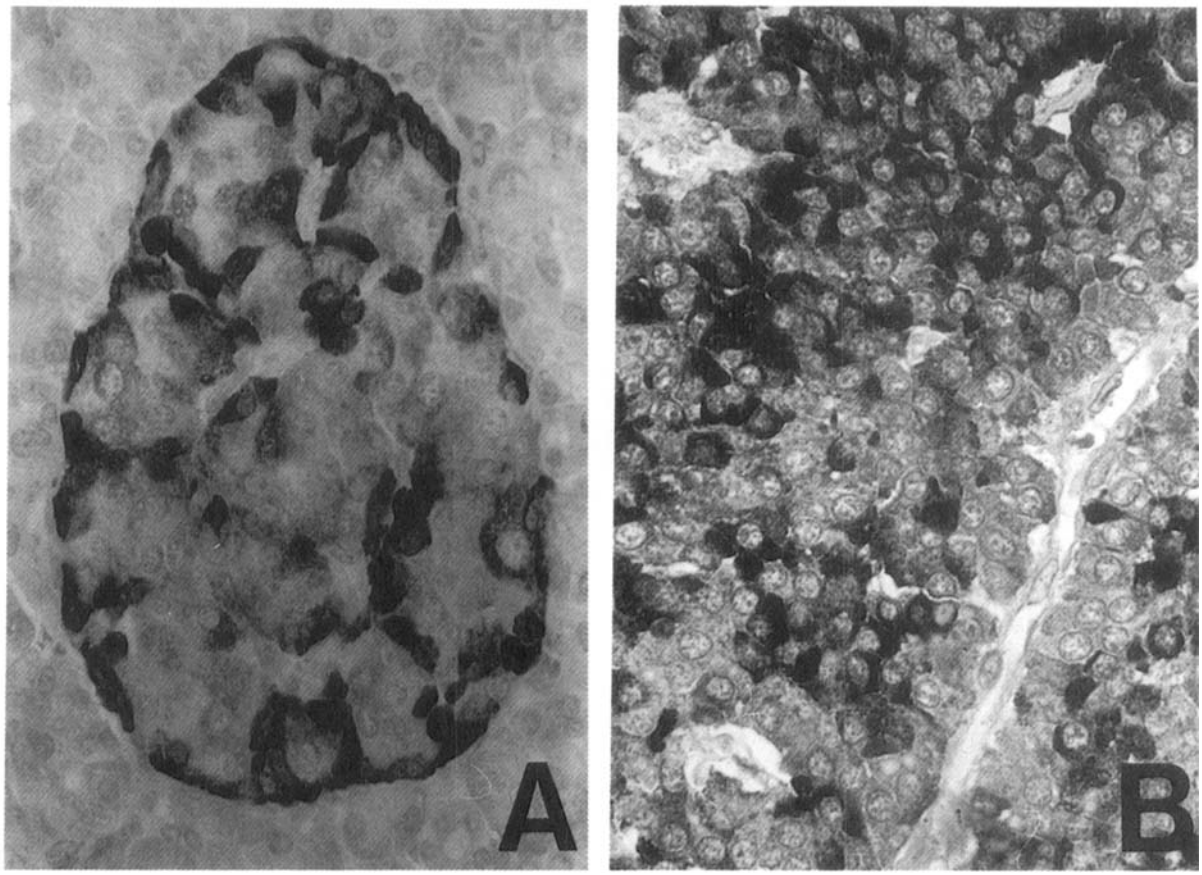

Fig. 3. Immunocytochemical localization of PLI in human tissue. Sections were immunostained with a C-terminal porcine pancreastatin antiserum. (A) Pancreatic islet of Langerhans, mainly staining of non-B-cells; (B) liver metastasis of a carcinoid tumor.

Biological activity

Endocrine pancreatic secretion

In their initial report, Tatemoto et al. (1) demonstrated that porcine pancreastatin inhibited glucose-induced insulin secretion in the isolated perfused rat pancreas. These observations were extended by Efendic et al. (45) who reported that pancreastatin reduced mainly the first phase of insulin secretion from the isolated perfused rat pancreas at 1 and 10 nmol/l, whereas the arginine-induced glucagon release was augmented. Higher concentrations (100 nmol/l) were needed to suppress glucose-stimulated insulin release from isolated rat pancreatic islets. Other authors confirmed that porcine pancreastatin inhibits insulin release induced by various stimuli such as arginine, glucose, inhibitors of phosphodiesterase (IBMX) or sulfonylureas (46–48). In mice, basal glucagon levels were augmented in vivo (47, 49).

Schmidt et al. (50) and Peiro et al. (51) demonstrated that porcine pancreastatin also inhibited insulin release in the perfused rat pancreas in response to gastrointestinal insulinotropic peptides, such as glucose-dependent insulinotropic polypeptide (GIP), vasoactive intestinal polypeptide (VIP) or cholecystokinin-8 (CCK-8). Somatostatin or glucagon release were not affected (51). Surprisingly, pancreastatin stimulated rather than inhibited dose-dependently insulin secretion from cultured adult rat islet cells in the presence of 4.2 mmol/l glucose, whereas inhibition was observed at high glucose levels (52). Human pancreastatin was also shown to inhibit glucose-stimulated insulin secretion from isolated rat pancreatic islets, although at pharmacological doses (100 nmol/l), the porcine equivalent being equipotent (53). In RIN m5F rat insulinoma cells, pancreastatin inhibited carbachol but not forskolin- or GIP-stimulated insulin release (54). These authors demonstrated that pretreatment of cells with pertussis toxin abolished this inhibition, indicating that in contrast to isolated islets and the perfused pancreas a cAMP-independent pathway might be involved.

The insulin-inhibitory effect is not restricted to the chromogranin A-derivative pancreastatin. Greeley et al. (55) reported that purified bovine parathyroid chromogranin A

(= secretory protein-I) dose-dependently inhibited insulin release from the isolated perfused rat pancreas. Since this effect was observed at a concentration of 1 nmol/l which is close to physiological circulating levels, it can be speculated whether chromogranin A itself is involved in the regulation of the endocrine pancreas.

What is the effect of porcine pancreastatin on endocrine pancreatic secretion in other species? The peptide showed no effect on glucose-induced insulin secretion from the in situ perfused canine pancreas (56). Insulin release in response to arginine or theophyllin was slightly increased. In contrast, intravenous administration of porcine pancreastatin inhibited CCK-8-induced insulin secretion in dogs in vivo, but had no effect on basal or glucose-induced insulin release (57). These controversial results are complemented by Holst et al. (58) who reported that porcine pancreastatin has no effect on endocrine secretion from the isolated perfused pig pancreas. In a careful study they demonstrated that neither basal nor glucose-stimulated secretion of insulin, glucagon and somatostatin release were influenced by pancreastatin at concentrations of 10 and 100 nmol/l.

The effect of pancreastatin on insulin and glucagon release in conscious rats was studied by Funakoshi et al. (59). Intravenous infusion of 1 and 10 nmol/kg/h of porcine pancreastatin inhibited plasma insulin and increased the plasma glucose response to the intragastric administration of glucose. Arginine-stimulated glucagon release was augmented. Funakoshi et al. (60) were the first to evaluate intra-species the effect of rat pancreastatin as a C-terminal 26-residue peptide in conscious rats. They demonstrated equipotency, compared to the porcine analogue, on glucose-stimulated insulin secretion. Sanchez et al. (61) reported that porcine pancreastatin (300 pmol/kg) produced a decrease in glycogen content of the liver and a slight hyperglycemia without affecting basal insulin or glucagon levels. Intracranial infusion of pancreastatin elevated dose-dependently blood glucose, fatty acids and corticosterone levels (62).

Exocrine pancreatic secretion

The bioactivity of pancreastatin on the exocrine pancreas was investigated under various conditions. Human pancreastatin [1–52] and its C-terminal fragment [24–52] inhibited CCK-stimulated pancreatic protein secretion in conscious rats with equal potency (63). Porcine pancreastatin exhibited the same activity. Ishizuka et al. (64) reported that porcine pancreastatin inhibited CCK-8 induced amylase release from dispersed guinea pig acini, whereas basal secretion was unaffected. A contrary result was found in isolated rat pancreatic acini, where the same compound was unable to suppress CCK-8 stimulated enzyme secretion (65), but did inhibit pancreatic protein and fluid secretion, stimulated by bile-diversion. Plasma CCK concentrations remained unchanged. To investigate whether species differences in the pancreastatin molecule could account for some of these discrepancies, porcine, bovine, human and rat pancreastatins were compared in their activity to inhibit pancreatic protein and fluid secretion in conscious rats (66). All 4 mammalian pancreastatins seemed to exert the same biological potency with regard to CCK-stimulated enzyme release. Nakano et al. (22) reported similar results for bovine pancreastatin. Miyasaka et al. (67) extended structure-activity studies for pancreastatin and demonstrated that the C-terminal amide structure of rat pancreastatin is required for the inhibitory activity on exocrine pancreatic secretion. The same group showed that rat pancreastatin dose-dependently inhibited CCK-8-stimulated pancreatic juice flow and protein output in vivo, whereas basal secretion and enzyme release from isolated acini were unaffected (68). Porcine pancreastatin was shown to inhibit postprandial exocrine pancreatic secretion in conscious rats, meal-stimulated insulin and gastrin plasma levels were not influenced (69). This study was the first to demonstrate an inhibitory effect of pancreastatin on the exocrine pancreas after physiological stimulation.

Gastric secretion

Lewis et al. (70, 71) investigated the influence of the C-terminal porcine pancreastatin [33–49] fragment on gastric acid secretion and somatostatin release in rabbit isolated parietal cells. Pancreastatin inhibited histamine- and carbachol-stimulated, but not forskolin-induced aminopyrine uptake dose-dependently. Somatostatin secretion remained unchanged. In contrast to its effect on rat insulinoma cell, the inhibitory influence of pancreastatin on the histamine stimulation of the parietal cell could be blocked by pertussis toxin, whereas the carbachol effect was not reversed. These studies seem to indicate that pancreastatin may interfere with different signal transduction pathways. In contrast, Hashimoto et al. (72) demonstrated that porcine pancreastatin [1–49] enhanced peptone meal-, phenylalanine- and glucose-stimulated gastric acid secretion in conscious dogs. Basal and histamine-induced gastric acid secretion or gastrin release were not influenced.

Other biological activities

Fasciotto et al. (73) reported that porcine pancreastatin inhibited chromogranin A and parathormone release from dispersed porcine parathyroid cells in culture in the presence of low calcium concentration. Phorbol-stimulated PTH release was also inhibited indicating that the activity of pancreastatin is calcium-independent. No effect was found on camostate-induced rat pancreatic hypertrophy which is mediated via release of endogenous CCK (74).

Porcine pancreastatin [1–49] and the C-terminal fragment [33–49] were reported to enhance memory retention after peripheral administration of the peptide in mice via an undefined mechanism (75).

Is pancreastatin a regulatory peptide?

In the light of controversial experimental results and a variety of divergent biological or pharmacological effects it is difficult to assess the physiological role of pancreastatin-like peptides. From several lines of evidence it is now firmly established that pancreastatin arises from proteolytic cleavage of its precursor chromogranin A, a member of the chromogranin/secretogranin family of proteins, present in secretory granules of endocrine and neuronal cells. Although isolated already more than 20 years ago (76), the function of these acidic proteins remains ill-defined. Several proposals include: a) a role in the organization and condensation of the granule matrix via its calcium-binding activity, b) modulation and inhibition of processing enzymes for peptide hormone and neuropeptide precursors, c) a regulatory function as hormonal proteins after secretion, d) precursor for regulatory peptides like pancreastatin (for review see ref. 8, 14, 15, 77, 78).

Several unusual features can be identified in an attempt to evaluate the physiological function of pancreastatin as a putative regulatory peptide hormone: The average sequence identity between pancreastatins from different species (porcine, bovine, human, rat) amounts to 70% (13, 14, 16). This represents a surprisingly low degree of sequence homology, when compared with other established gut hormone peptide families. Furthermore, it can be noticed that other regions in the N-terminal and C-terminal part of the chromogranin A sequence show close to 100% structural identity, thus questioning the hypothesis that the main function for chromogranin A is to release pancreastatin as a regulatory circulating hormone. The proteolytic processing of chromogranin A is still incompletely understood: Although release of a pancreastatin-like peptide from the perfused porcine pancreas has been demonstrated (40), the molecular nature of other chromogranin A derived peptides, released into the circulation or present in tissues, is unknown.

To classify pancreastatin as a novel peptide hormone with physiological relevance for the regulation of the endocrine and exocrine pancreas, a number of crucial requirements are still not fulfilled: According to the criteria set up by Grossman (79) and Rehfeld (80), it has not been demonstrated so far that infusion of exogenous pancreastatin at a dose that produces physiological postprandial plasma levels, provoked any biological effect. Most of the actions of pancreastatin were observed at pharmacological doses, often with peptide material from a species other than the biological assay system, and none of these studies reported whether plasma levels reached by their infusion regimen corresponded to physiological postprandial plasma concentrations. Few examples of pancreastatin plasma levels were in the 10^{10} mol/l range and showed unsatisfactory elevation after meal stimuli (35). This would be no argument against a physiological role, if a peptide has mainly or exclusively a paracrine or even an autocrine function (81). However, no pancreastatin-specific high-affinity receptors have been identified, despite the fact that pancreastatin is now 4 years old. We were unable to identify pancreastatin binding sites on RIN m5F rat insulinoma cells (W. E. Schmidt & B. Gallwitz, unpublished observation), which were reported to respond to pancreastatin (54). These findings argue against a role for pancreastatin in the regulation of insulin release and exocrine pancreatic secretion. It is of crucial importance to look for the existence of high affinity pancreastatin receptors in all tissues and cell types which have been used as a biological screening assay to elucidate biological activities of pancreastatin.

Is pancreastatin a classical regulatory peptide? The answer is probably 'no'. What might be the role of this peptide, of other chromogranin A-derived fragments and of chromogranin A itself? If no receptors for this peptide on target cells can be demonstrated, a role as hormone, paracrine substance or neurotransmitter can be ruled out. Then, the question arises whether pancreastatin exerts its main action intracellularly, as a component of the granule protein matrix that could modulate processing enzyme activity and play a role during the course of hormone biosynthesis. This hypothesis needs further investigation. It includes the assumption that the diverse biological effects of pancreastatin are due to a mechanism which does not require a specific hormone-receptor interaction. Chromogranin A has been shown to bind calcium. It can be speculated that pancreastatin as a high-negatively charged molecule might be able to interfere with ion channels, synaptic transmission or free ions directly and thereby act more as a pharmacological agent than like a true endogenous modulator. A number of examples exists where random peptide fragments, derived from hemoglobin or actin, exhibited a certain biological activity (82, 83). Therefore, it is important to elucidate the mechanism of action for this peptide and characterize its biological effects under strictly physiological conditions, before pancreastatin can be added to the steadily growing family of 'true' regulatory peptides.

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