REVIEW

Pancreastatin: further evidence for its consideration as a regulatory peptide

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ABSTRACT

Pancreastatin is a 49 amino acid peptide first isolated, purified and characterized from the porcine pancreas, and whose biological activity in different tissues can be assigned to the C-terminal part of the molecule. Pancreastatin has a prohormonal precursor, chromogranin A (CGA), which is a glycoprotein present in neuroendocrine cells, including the endocrine pancreas. Both intracellular and extracellular processing of CGA can yield pancreastatin. This processing is tissue-specific, with the pancreatic islet and antral gastric endocrine cells being the major source of fully processed pancreastatin. Most of the circulating CGA is secreted by chromaffin tissue. Therefore, peripheral processing of CGA is probably the major indirect source of pancreastatin. Pancreastatin seems to have a general modulatory control on endocrine (insulin, glucagon, parathormone) and exocrine (pancreatic, gastric) secretion from tissues close to the source of production. This has led to the assumption that pancreastatin may be a peptide with an autocrine and paracrine function. It has recently been revealed to be a peptide with a metabolic function counter-regulatory to insulin action. This effect, in conjunction with the inhibitory effect on insulin and pancreatic exocrine secretion, points to a role in the physiology of stress. The molecular mechanism of the glycogenolytic effect of pancreastatin is better known, although further work is still needed. In general, more studies should be carried out at the molecular level to investigate the mechanism of action of pancreastatin and thus to clarify its physiological role in the neuroendocrine system.

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Introduction

Pancreastatin (PST), a 49 amino acid peptide with a carboxy-terminal glycaminide, was first isolated, purified and characterized from the porcine pancreas by Tatemoto et al. (1986), ten years ago. Its role as a regulatory enteropancreatic hormone has been established because of a variety of biological effects in a number of tissues; effects that may be assigned to the carboxy-terminal part of the molecule (Schmidt & Creutzfeldt 1991). Although the molecular mechanisms of the action of PST for most of the effects so far described remain elusive, we are now closer to understanding the possible role of this peptide.

It is now established from several lines of evidence that PST arises from proteolytic cleavage of its precursor chromogranin A (CGA) (Eiden 1987, Huttner & Benedum 1987), a glycoprotein present in endocrine and neuronal cells. Its amino acid sequence of 240–288 in porcine CGA corresponds to that in PST flanked by typical signals for proteolytic processing (Iancangelo et al. 1986, 1988a, Konecki et al. 1987, Helman et al. 1988).

In the present review we have compiled the different effects of PST that have been described as well as some clues about its mechanism of action. We have also tried to draw a picture of the putative role that PST may play in the general physiology of neuropeptides, in a model that still needs to be demonstrated.

The general inhibitory action of PST on endocrine and exocrine secretion led to the assumption that PST might play a role in the fine
regulation of secretion at the paracrine, autocrine and endocrine levels. However, the relationship between PST and cromaffin tissue and the counter-regulatory effect of PST on insulin action that we have described (Sánchez-Margalef & Gobena 1994a), as well as the inhibitory effect on insulin and exocrine pancreatic secretion, have suggested the hypothesis that PST could also play a role in the physiology of stress (Sánchez-Margalef & Gobena 1993c).

The physiological role of PST still remains to be clarified, however.

**Molecular forms**

PST was first isolated from the porcine pancreas as a 49 amino acid peptide amide (Tatemoto et al. 1986). Human PST is deduced to be a 52-residue peptide amide corresponding to CGA 250–301 from the gene structure of human CGA which is homologous to porcine PST (Konecki et al. 1987). Bovine PST is a 47-residue peptide amide which has been isolated from pancreas and pituitary (Nakano et al. 1989), and it has turned out to be also identical to bovine CGA cDNA. Rat CGA cDNA also revealed a PST-like sequence homologous to porcine PST (Hutton et al. 1988, Iancangelo et al. 1988b, Parmer et al. 1989, Aboud & Eberwine 1990). The homology in the peptide sequence of human PST with other species ranges from 75% with porcine PST to 55% with rat PST.

Different molecular forms of PST have been isolated from human tumors, comprising 29 (273–301 CGA), 48 (254–301 CGA), 92 (210–301 CGA) and 186 (116–301 CGA) amino acid residues (Schmidt et al. 1988, Sekiya et al. 1988, Funakoshi et al. 1989a, Tamamura et al. 1990). Different phosphorylated forms of PST have been reported depending on the localization of the peptide (bovine pancreas or ileum) (Watkinson et al. 1993). However, the circulating forms of PST are more important physiologically. Thus, human PST-52 and a larger form of 15–21 kDa have been shown to be the major molecular forms in normal human plasma (Kitayama et al. 1994). Interestingly, both seem to have biological activity. Whether the larger molecular form corresponds to PST-186 remains to be confirmed.

**Source of PST**

CGA, the precursor for PST as well as for other peptides, is widely distributed throughout cells of the neuroendocrine system (Simon & Aunis 1989), and is the major catecholamine storage vesicle soluble protein (O’Connor & Frigon 1984). Neuroendocrine cell-specific expression seems to be mediated by a consensus cyclic AMP (cAMP) response element in the CGA gene (Moulard et al. 1994).

Unprocessed CGA appears to be the major storage product in secretory vesicles of adrenal medulla, hypothalamus and anterior pituitary (Kar et al. 1989), although it is generally accepted that degradation products of chromogranins exist in chromaffin cells (Simon & Aunis 1989), probably degraded by calcium-dependent proteases (Settleman et al. 1985). The primary amino acid sequence of CGA contains several conserved paired basic residues (Iancangelo et al. 1991, Wu et al. 1991) which constitute potential sites of cleavage.

The processing of CGA is tissue-specific (Winkler & Fischer-Colbrie 1992). Thus, CGA processing is more extensive in endocrine cells of the gut and pancreas (especially in gastric antral endocrine cells and pancreatic islets), where PST is one of the major conversion products (Watkinson et al. 1991) that are also present in the secretion (Börglum-Jensen et al. 1994).

In islets, PST appears to be localized to the insulin-containing β cells, somatostatin-containing δ cells (Ravazzola et al. 1988) and glucagon-containing α cells (Curry et al. 1990, Lamberts et al. 1990). The post-translational processing of CGA in the pancreatic islet involves the subtilisin-related protease PC2 (Arden et al. 1994). On the other hand, post-secretory processing of CGA does occur (Simon et al. 1989, Watkinson et al. 1990). This processing may be due to proteolytic enzymes localized in secretory granules in an inactive form and released together with catecholamines and chromogranins on stimulation. Alternatively, active exoproteases may be localized on the extracellular side of cell plasma membranes, as previously hypothesized (Metz-Boutigue et al. 1993). Moreover, fragmentation of bovine CGA by plasma kalikrein has been shown (Leduc et al. 1990).

Since most of the circulating CGA originates in chromaffin tissue, this origin may be the major indirect source for the production of circulating PST. With regard to the secretion of fully processed PST, the endocrine pancreas and gastric antral endocrine cells seem to be the major source.

Plasma porcine PST-like immunoreactivity (PST-LI) levels have been shown to increase 50% (from 100 pm to 150 pm) in response to a meal (Bretherton-Watt et al. 1988). In perfused porcine pancreas, PST-LI is released in parallel with insulin in response to insulinotropic stimuli (Östenson et al. 1989a). In perfused isolated porcine antrum, 1–49 PST is the major secreted form although larger molecular weights are also secreted upon electrical
stimulation of vagal nerves (Börglum-Jensen et al. 1994). In the non-antral stomach, larger molecular forms predominate in the secretion after vagal stimulation (Börglum-Jensen et al. 1994). In humans, PST-LI is increased in response to the intrajejunal infusion of a liquid mixed meal (Funakoshi et al. 1990a). Secretion of PST-LI has also been reported in a human somatostatin-producing cell line (QGP-1N) (Funakoshi et al. 1990b, 1993), where it is regulated by acetylcholine involving a pertussis toxin non-sensitive G protein (Funakoshi et al. 1991, 1992, Tateishi et al. 1992a).

Elevated PST-LI levels have been found in response to glucose loading in non-insulin-dependent diabetes mellitus (Funakoshi et al. 1990c) and non-obese subjects with essential hypertension, following catecholamine secretion (Sánchez-Margalet & Goberna 1993c, Sánchez-Margalet et al. 1995a).

The kidney and liver must play a role in the elimination of PST, since there are elevated PST-LI circulating levels in patients with chronic renal failure and liver cirrhosis (Tateishi et al. 1989). Moreover, degradation of PST by human kidney extract has been reported (Tateishi et al. 1992b), and PST-LI can also be found in urine (Tateishi et al. 1990).

Biological activity

Endocrine pancreatic secretion

PST was initially described as an inhibitor of glucose-induced insulin secretion in the isolated rat pancreas (Tatemoto et al. 1986). This effect is exerted mainly in the first phase of insulin secretion (Efendic et al. 1987). We have also found an inhibitory effect on the first phase of insulin secretion in the perfused isolated rat pancreas, although PST enhanced the priming effect of glucose after a second pulse of glucose (Sánchez-Margalet & Goberna 1993a). On the other hand, PST can also stimulate insulin secretion from cultured islet cells (Ishizuka et al. 1988) and increases cytosolic Ca²⁺ in insulin-secreting RIN m5F cells (Sánchez-Margalet et al. 1992b).

PST can inhibit many insulinotropic stimuli: peptides (gastric inhibitory peptide, vasointestinal peptide and cholecystokinin (CCK-8)) (Peiró et al. 1991), arginine, isobutyryl-methylxanthine (Silvestre et al. 1988, Östenson et al. 1989), carbacchol (Lorinet et al. 1989) and glucagon (Sánchez-Margalet et al. 1992a). The inhibitory effect of PST on insulin release has been confirmed in vivo studies in the rat (Funakoshi et al. 1989b,c, Sánchez-Margalet et al. 1992a). However, in the dog, there are controversial results in vivo: PST had no effect on glucose-induced insulin release (Ohneda et al. 1989), and even slightly increased the insulin secretion stimulated by arginine or theophyllin, although it inhibited CCK-8-stimulated insulin release (Inui et al. 1989). In the isolated perfused pig pancreas, no effect of PST on endocrine secretion has been reported (Holst et al. 1990).

Glucagon secretion seems to be stimulated by PST both in vitro (Efendic et al. 1987) and in vivo (Ahren et al. 1988, Funakoshi et al. 1989b,c).

Exocrine pancreatic secretion

PST has an inhibitory effect on the exocrine pancreas. This effect has been investigated in rats in vivo after physiological stimulation (Miyasaka et al. 1989), stimulation with CCK-8 (Miyasaka et al. 1990a,b), and central vagal nerve stimulation (Miyasaka et al. 1990c). In vitro, PST also suppresses CCK-8-stimulated enzyme secretion (Ishizuka et al. 1989), although contrary data have been reported using a different experimental model (Funakoshi et al. 1989d).

Similar to biological activity on endocrine pancreatic secretion, the C-terminal amide structure of PST seems to be required for the inhibitory activity on exocrine pancreatic secretion (Miyasaka et al. 1990a). The inhibitory effect of PST on pancreatic enzyme secretion seems to be mediated by presynaptic modulation of acetylcholine release (Herzig et al. 1992). Therefore, it has been suggested that PST is a mediator in the islet-acinar axis (Von Schonfeld et al. 1993).

Gastric secretion

Different effects of PST on gastric secretion have been reported, depending on the experimental model. In vitro, PST inhibits gastric acid secretion in rabbit isolated parietal cells (Lewis et al. 1988, 1989). However, in vivo, PST seems to enhance gastric acid secretion in conscious dogs when stimulated by a peptone meal, phenylalanine or glucose (Hashimoto et al. 1990).

Parathyroid hormone secretion

PST is also a negative regulator of parathormone release. Thus, PST inhibits parathyroid hormone (PTH) secretion stimulated by physiological (a low calcium concentration) and non-physiological (phorbol) stimulators in porcine (Fasciotto et al. 1989) and bovine (Drees & Hamilton 1992) parathyroid cells. PTH secretion can also be enhanced by the incubation of parathyroid cells with anti-PST antibodies (Fasciotto et al. 1990). PST inhibits not only the secretion but also the transcription of the parathyroid hormone and CGA genes and decreases the stability of the respective
mRNAs in parathyroid cells in culture (Zhang et al. 1994). However, the specific mechanism by which PST effects changes in transcription are not known.

Glycogen metabolism
We have shown that PST has a glycogenolytic effect in the rat liver. Glucose release from hepatic glycogen was increased in the rat in vivo, resulting in a hyperglycemic effect (Sánchez et al. 1990, Sánchez-Margalet et al. 1992c). This effect was confirmed in isolated rat hepatocytes in vitro (Sánchez et al. 1992, Sánchez-Margalet et al. 1993), and was found to be dependent on the presence of calcium in the incubation media. Moreover, PST inhibits insulin-stimulated glycogen synthesis in vitro (Sánchez-Margalet & Goberna 1994a). On the other hand, the rate of glycolysis in insulin-stimulated hepatocytes was not inhibited by PST. These data suggest that PST could have a direct effect on liver as a counter-regulatory peptide of insulin action.

The glycogenolytic effect of PST is comparable with that exerted by glucagon, although the latter produces a more pronounced hyperglycemia. The administration of PST (300 pmol/kg) in addition to glucagon does not increase hepatic glycogenolysis, which is already maximal, but inhibits glucagon-stimulated insulin release and, in this way, enhances the hyperglycemic effect of glucagon (Sánchez-Margalet et al. 1992a).

Other biological activities
PST has been shown to enhance memory retention after peripheral administration of the peptide in mice (Flood et al. 1988).

We found that, in vivo, PST decreases catecholamine levels in the rat under surgical stress (Sánchez-Margalet & Goberna 1993b), suggesting a possible role modulating chromaffin secretion.

PST has no effect on carbachol-induced rat pancreatic hypertrophy which is mediated via release of endogenous CCK (Schmidt et al. 1989).

Mechanisms of action
In spite of the fact that PST has been shown to have a variety of biological effects in a number of tissues, little is known about its exact mechanisms of action.

PST-specific high-affinity receptors have been identified in rat liver membranes by our group (Sánchez-Margalet et al. 1994a). That study suggested that the high-affinity PST receptor exists as a monomeric glycoprotein with an apparent molecular weight of 35 000 and that it is functionally coupled to a GTP-binding protein which is pertussis toxin-insensitive, as indirectly determined by the inhibition of PST binding to the membranes by guanine nucleotides and the GTPase activity stimulated by PST (Sánchez-Margalet et al. 1994a). Analysis of binding under equilibrium conditions indicated the existence of one class of binding sites, with a Bmax of 15 fmol/mg protein and a Kd of 0-2 nm. Knowledge about the glycoprotein nature of the PST receptor in liver membranes may be useful for its purification in future work. No PST-specific receptors have been found in any other tissue so far.

The physiological relevance and molecular mechanisms of other biological activities remain, therefore, to be elucidated. However, there is indirect evidence for the involvement of a GTP-binding protein in the inhibitory effect of PST in the RIN m5F rat insulinoma cell line, since it is abolished by pretreatment of the cells with pertussis toxin (Lorinet et al. 1989). The inhibitory effect of PST on histamine-stimulated parietal cells is also blocked by pertussis toxin (Lewis et al. 1989), although the inhibitory effect on carbachol stimulation was not reversed, indicating that PST may interfere with different signal transduction pathways. Whether PST is interacting directly with a G protein or whether this interaction is mediated by a specific receptor in the plasma membrane remains to be ascertained.

We have studied the signal transduction of the PST receptor in the hepatocyte. The glycogenolytic effect of PST is dependent on calcium but not on cAMP (Sánchez et al. 1992). Moreover, we have shown that PST increases intracellular calcium, involving both pertussis toxin-sensitive and -insensitive mechanisms (Sánchez-Margalet et al. 1993). The mobilization of intracellular calcium is dependent on the production of inositol 1,4,5-triphosphate (IP3) through a pertussis toxin-insensitive mechanism, whereas the stimulation of calcium inflow involves a pertussis toxin-sensitive mechanism. In fact, PST increases the production of IP3 and diacylglycerol (DAG) in liver membranes (Sánchez-Margalet & Goberna 1994b) by activating phospholipase C (PLC) activity. The production of DAG is responsible for the activation of protein kinase C (PKC) by PST (Sánchez-Margalet et al. 1994b) as would be expected (Nishizuka 1984). The production of IP3 and DAG (Berridge 1987) are two synergistic limbs of the phosphotidylinositol biphosphate signaling pathway in the hepatocyte (Exton 1988), since some targets of Ca2+-calmodulin-dependent phosphorylation are also targets for PKC (e.g. glycogen synthase).

PST also increases the production of cGMP involving a pertussis toxin-sensitive G protein (Sánchez-Margalet & Goberna 1994b).
Physiological role of cGMP in liver metabolism is not known. However, there is experimental evidence that the production of cGMP mediates the inhibition of PLC activity stimulated by PST (Sánchez-Margalet & Goberna 1994b). Therefore, cGMP could function as a negative feedback or down-regulation in the signal transduction of the PST receptor.

Physiological implications

In the light of the actions of PST that have been characterized, this peptide seems to behave as a negative autocrine and/or paracrine regulator of endocrine and exocrine secretion. Thus, the PST produced and released by the pancreatic islets and gastric antral endocrine cells exerts its effect in a nearby gland: islet endocrine cells, exocrine pancreas and gastric mucosa. In fact, PST could be one of the mediators of the islet-acinar axis (Von Schonfeld 1993).

On the other hand, there are two examples of the regulation of the gland’s secretion as an endocrine agent: the parathyroid gland and the adrenal medulla. In both cases, CGA rather than PST seems to be the major secretion product and therefore this peptide could exert its inhibitory action at these levels in a long-loop negative feedback only after peripheral degradation of CGA to yield PST.

The metabolic effect of PST on the hepatocyte is the best example of its role as an endocrine peptide: the PST released by pancreatic islets or the PST produced by postsecretory processing of CGA secreted from chromaffin cells is acting in a target tissue (the liver) far from the place of production. This metabolic effect fulfills all the criteria for an endocrine agent, i.e. it is exerted at a low concentration that occurs in circulating levels in vivo; this effect is dose-dependent and is mediated by a specific receptor coupled to an effector system, the activation of PLC.

The overall inhibitory effect of PST on endocrine and exocrine secretion, in conjunction with the putative counter-regulatory effect of insulin in glucose metabolism, points to a physiological role in the response to stress. There are no reports describing an increase of PST secretion in hypoglycemia or any other stressful situations so far. However, PST has been shown to correlate with CGA in patients with neuroendocrine neoplasia (Syversen et al. 1993, 1994) and CGA release is known to be increased in stressful conditions following catecholamine secretion (Cryer et al. 1991, Dimsdale et al. 1992). In addition, PST levels seem to correlate with norepinephrine in essential hypertension (Sánchez-Margalet et al. 1995b). Therefore one could expect that PST levels would follow the increase of catecholamine levels during stress, although this hypothesis remains to be tested. In this context, we may speculate that PST could help catecholamines in their action on glucose metabolism to provide glucose to muscles and the brain. Moreover, if these effects are confirmed, PST could well play a role, not only in the physiology of glucose metabolism, but also in pathophysiological conditions such as diabetes mellitus and other insulin-resistant states, such as hypertension, as has previously been hypothesized (Funakoshi et al. 1990c, Sánchez-Margalet & Goberna 1993c, Sánchez-Margalet et al. 1995a,b).

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