Chromogranin A-derived peptides: functional aspects of vasostatins, pancreastatin, catestatin and parastatin.

Karen B. Helle[a], Sushil K. Mahata[b]

a. Section of Physiology, Department of Biomedicine, University of Bergen, Norway.
b. Department of Medicine, University of California, San Diego, USA.

Correspondence: Dr. KarenB.Helle, Section of Physiology, Department of Biomedicine, University of Bergen, Jonas Lies vei 91, 5009 Bergen, Norway, **Phone:** 47-55-586416; **Fax:** 47-55-586360, **Email:** karen.helle@fys.uib.no

Cell Biology of the Chromaffin Cell
R. Borges & L. Gandía Eds.
Instituto Teófilo Hernando, Spain, 2004

The occurrence of chromogranins and their derived peptides in the extracellular space is a prerequisite for their function as prohormones for regulatory peptides. The effects may be multifaceted, autocrine, paracrine and/or endocrine, acting at concentrations that may reflect the distance from the site of release to their target site. Local concentrations of the chromogranins and their processed products may reflect autocrine and paracrine effects while transport across the vascular endothelium into the circulation would be required for endocrine effects. The first reported target for a CgA-derived peptide was the porcine pancreatic beta cells and accordingly the peptide was named pancreastatin.[1] During the last decade the number of targets for CgA-derived peptides have increased substantially.[2] The aim of this "Wine & Beer" session was to focus on recent findings of effectors for the main CgA-derived peptides vasostatins, pancreatatin, catestatin and parastatin (Fig. 1), to gather insight into mechanisms activated by the multitude of activities currently assigned to these peptides.

The modulatory activity of CgA peptides in different models of hormone secretion was discussed by Maurizio Mandalà, reporting that catestatin, inhibiting catecholamine release in adrenomedullary cells[3] while stimulating histamine release in rat mast cells[4], was completely without modulating effects on the secretory process in the human blood platelets. He also reported on a selective interaction between the smooth muscle layer of the rat posterior cerebral artery and the cationic bovine $CgA_{47\text{-}70}$ and chromofungin ($CgA_{47\text{-}66}$).

Vasostatins (VSs) as inhibitors of myocardial inotropy in vertebrate hearts. Bruno Tota, reported data consistent with a role for VS-1 as cardiosuppressive, counter-adrenergic peptides in vertebrates, extending the inhibitory role of VSs in the vascular system[5] to that of the heart[6]. In the eel (*Anguilla anguilla*) the VS-mediated negative inotropism required an intact endocardial endothelium and involved Gi/o proteins, muscarinic and adrenergic receptors, calcium channels and the NO-cGMP-PKG pathway[7]. VS-1 also counteracted the classical inotropic response to adrenergic stimulation[7]. In the frog (*Rana esculenta*) heart the negative inotropism of VS involved neither the Gi/o proteins nor the NO-cGMP system while required the activation of K^+ channels.[8] In the Langendorff preparation of the rat heart, VS-1, but not VS-2, decreased the inotropism without changing

coronary pressure. Both peptides counteracted the positive inotropism mediated by adrenergic stimulation.[9]

Pancreastatin (PST) as a regulator of energy metabolism. Víctor Sánchez-Margalet reported that in rat adipocytes and hepatocytes the metabolic actions of PST are mediated by specific receptors, resulting in activation of the effector system $G_{q/11}$-PLC-β-PKC-MAPK. [10, 11] In these cells PST has a counter-regulatory effect on insulin signalling and action, inhibiting glucose uptake, glycogen and lipid synthesis as well as promoting lipolysis and also a negative cross-talk with insulin receptor signalling.[12] More recently PST inhibited leptin secretion by decreasing leptin expression in isolated rat adipocytes whereas UCP-2 expression was upregulated. This suggested that PST may modulate energy metabolism by both direct and indirect mechanisms.

Catestatin as a multifunctional peptide with antimicrobial activity. Marie-Hélène Metz-Boutigue reported for the first time that the arginine rich N-terminus of catestatin ($CgA_{344-358}$) was a potent inhibitor of microbial growth, not only of Gram-positive bacteria such as *Micrococcus luteus* and *Bacillus megaterium* (MIC 0.8 μM), but also of pathogenic Gram-negative bacteria such as *Escherichia coli D22* (MIC 8 μM), a range of filamentous fungi and several candida yeast cells (MIC 0.2–10 μM). In contrast, the N-terminal core of catestatin was unable to kill erythrocytes at the concentration range effectively inhibiting microbial growth. These findings were consistent with the hypothesis that the cationic and hydrophobic domains of CgA, chromofungin[13] and catestatin[4], may interact with biological membranes in a receptor-independent manner, analogous to that of other cationic and amphipathic peptides with antimicrobial potencies[14].

The N-terminus of parastatin (PARA) and para-related peptides. The role of these peptides, acting as autocrine inhibitors of porcine parathyroid secretion, was discussed by Brigitte H. Fasciotto Dunn. In the parathyroid cells CgA and parathormone are co-stored and co-secreted upon stimulation by hypocalcemia, yet subject to autoinhibition[15], possibly by three naturally occurring PARA

peptides[16]. Blocking of furin-mediated CgA processing[17] extends the functional concept of CgA-derived PARA peptides in autocrine inhibition of parathormone secretion that could account for the pattern of pulsatile parathormone release *in vivo*.

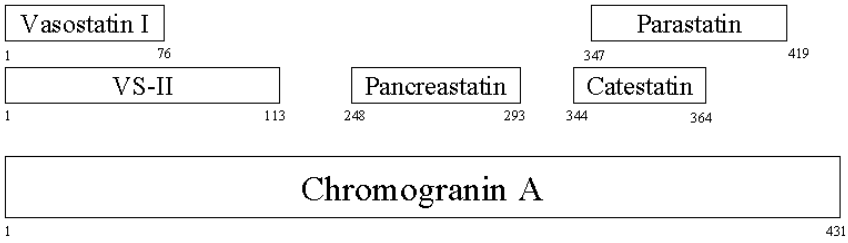

Figure. 1. Schematic presentation of the primary structure of the bovine CgA and main peptides.

Catestatin mutants and hypertension Sushil K. Mahata reported on catestatin's antagonism of nicotinic-cholinergic stimulation of catecholamine secretion and CgA gene transcription in mice, establishing catestatin's autocrine/paracrine effects *in vivo*[18]. He also presented novel data on the discovery of naturally occurring human ($CgA_{352-372}$) catestatin variants ($Gly_{364}Ser$, $Pro_{370}Leu$, $Arg_{374}Gln$) and their differential effects on nicotine evoked catecholamine secretion[19]. $Gly_{364}Ser$ represents a change to an amino acid (Ser) not previously seen at this sequence position in any mammal; $Pro_{370}Leu$ is a reversion of the wild type human amino acid (Pro) to the amino acid (Leu) seen in all non-primate mammals; and$Arg_{374}Gln$ disrupts the usual dibasic processing site ($Arg_{373}Arg_{374}$) flanking the carboxy-terminus of catestatin[19]. In addition, he briefly discussed severe alterations in chromaffin cell physiology in CgA knockout mice implicating that catestatin plays a crucial role in the pathogenesis of hypertension.

SUMMING UP

The most recent findings strengthen the concept of CgA as a prohormone for at least four peptides with modulating potencies in a wide range of target systems. However, apart from the non-competitive inhibition of the nicotinic acetylcholine receptor by catestatin in

chromaffin cells, classical surface receptors for CgA peptides in the other target systems are still elusive. Nonetheless, involvement of G-protein subunits has been implicated in a wide range of cells and tissues, for vasostatins in mammalian blood vessels and fish heart, for pancreastatin in the rat adipocytes and hepatocytes and for catestatin in the rat mast cells. The potent antimicrobial activities of the cationic and amphipathic domains inherent in vasostatin I (chromofungin) and in catestatin, point to receptor-independent activation of intracellular signalling mediated via membrane interaction and penetration. The possibility of similar receptor-independent activations elicited by the vasostatins and by catestatin in target systems of mammalian origin provides a challenge for future approaches.

REFERENCES

1. Tatemoto, K., et al., *Pancreastatin, a novel pancreatic peptide that inhibits insulin secretion*. Nature, 1986. **324:**476-478.
2. Helle, K.B., et al.. *Chromogranin A as a calcium-binding precursor for a multitude of regulatory pepties for the immune, endocrine and metabolic systems.* Curr Med Chem - Immun., Endoc. & Metab. Agents, 2001. **1:**119-140.
3. Mahata, S.K., et al., *Novel endocrine feedback control of catecholamine release: a discrete chromogranin A fragment is a non-competitive nicotinic cholinergic antagonist.* J Clin Invest, 1997.**100:**1623-1633.
4. Krüger, P-G., et al., *Catestatin ($CgA_{344-364}$) stimulates rat mast cell release of histamine in a manner comparable to mastoparan and other cationic charged peptides.* Regul Pept, 2003. **114:**29-35.
5. Brekke, J.F., et al., *N-terminal chromogranin-derived peptides as dilators of bovine coronary resistance arteries*. Regul Pept, 2002. **105:**93-100.
6. Corti, A., et al., *Vasostatins exert negative inotropism in the working heart of the frog*. Ann NY Acad Sci, 2002. **971:**362-365.
7. Mazza, R., et al., *Cardiosuppressive action of vasostatins on the frog heart.* In 12 Int. Symp on Chromaffin Cell Biol, 2003. p 3-11.
8. Imbrogno, S., et al., *The inotropic influence of vasostatins on the working heart of the eel (Anguilla anguilla): paracrine aspects and subcellular mechanisms*. In 12 Int. Symp on Chromaffin Cell Biol, 2003. p 3-07.
9. Angelone, T., et al., *Cardiosuppressive action of vasostatins on the Langedorff isolated and perfused rat heart.* In 12 Int. Symp on Chromaffin Cell Biol, 2003. p 3-09.
10. Gonzales-Yanes, C., et al., *Characterization of pancreastatin receptors and signaling in adpocyte membranes.* Biochim Biophys Acta, 1999.**1451:**153-162.
11. Santos-Alvarez, J., et al., *Pancreastatin receptor is coupled to guanosine trihosphate-binding protein of the G(q/11) alpha family in rat liver membranes*. Hepatology, 1998. **27:**608-614.

12. Gonzales-Yanes, C. and V. Sanchez-Margalet, *Pancreastatin, a chromogranin A derived peptide, inhibits insulin-stimulated glycogen synthesis by activating GSK-3 in rat adipocytes.* Biochim Biophys Res Commun, 2003. **289:**282-287.
13. Lugardon, K., et al., *Antibacteial and antifungal activities of vasostatin-I, the N-terminal fragment of chromogranin A*. J Biol Chem, 2000. **275:**10745-10753.
14. Lugardon,K., et al., *Structural and biological characterization of chromofungin, the antifungal chromogranin A (47-66)-derived peptide.* J Biol Chem, 2001. **276:**35875-35882.
15. Fasciotto, B.H., S.U Gorr, and D.V. Cohn, *Autocrine inhibition of parathyroid cell secretion requires proteolytic processing of chromogranin A.* Bone Minerals, 1992.**17:**323-333.
16. Fasciotto, B.H., et al., *Processing of chromogranin A in the parathyroid generation of parastatin-related peptides.* Peptides, 2000. **21:**1389-1401.
17. Fasciotto, B.H., and D.V. Cohn, *Chromogranin A-derived parastatin peptides as autocrine inhibitors of endocrine secretion.* In 12 Int. Symp on Chromaffin Cell Biol, 2003. p -3-10.
18. Mahata, S.K., et al., *Catecholamine secretory vesicle stimulus-transcription coupling in vivo: demonstration by a novel transgenic promoter/photoprotein reporter, and inhibition of secretion and transcription by the chromogranin A fragment catestatin*. J Biol Chem, 2003. **278:**32058-32067.
19. Wen, G., S.K. Mahata, et al., *Both rare and common polymorphisms contribute functional variation at CHGA, a regulator of catecholamine physiology.* Am J Hum Genet, 2004. **74:**197-207.