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# THE CHROMOGRANIN A-DERIVED PEPTIDES: STRUCTURAL AND FUNCTIONAL FEATURES IN HEART BIOLOGY

[Peptidi derivati dalla cromogramina A: caratteristiche funzionali e strutturali relative alla biologia del cuore]

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**Key words**: Vasostatin I, Vasostatin II, Natural peptides, Neuroendocrine tissue, Heart physiology
**Parole chiave**: Vasostatina I, Vasostatina II, Peptidi naturali, Tessuto neuroendocrino, Fisiologia del cuore

**Abstract.** Chromogranin A (CGA) is a 48 kDa acidic protein co-stored in secretory granules of several endocrine and neuroendocrine cells with other compounds and it is co-released with them by exocytosis. Either inside the granules or in extracellular fluid or into circulation, CGA is cleaved by tissue-specific proteolytic enzymes into smaller peptides with different biological activities. High levels of plasma CGA have been correlated to endocrine and neuroendocrine tumours, renal failure and heart failure and it has been recently demonstrated that CGA is produced not only by the diffuse neuroendocrine cells but also by cells of different embryologic origins. Moreover, new effects of CGA-derived peptides come out every day.
The main CGA-derived peptides correlated to heart biology are Pancreastatin (PST) and Vasostatins (VSs). PST is a 49-amino-acid peptide, corresponding to bovine $CGA_{248-293}$ and human $CGA_{240-288}$, able to stimulate secretion in neonatal rat atrial cells. Vasostatin I (VS-I) and Vasostatin II (VS-II) correspond to the N-terminal fragments $CGA_{1-76}$ and $CGA_{1-113}$ respectively, and seem to be the best cardioregulatory peptides in mammals, since they counteract the β-adrenergic-mediated positive inotropism in vertebrate hearts. These findings let us suppose that CGA and CGA-derived peptides may play an autoregolatory role in heart secretion.
This review would be a summary of the possible relationships among CGA and CGA-derived peptides and heart physiology, biology and pathology.

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**Riassunto.** La cromogranina A (CGA) è una proteina acida di 48 kDa conservata nei granuli di secrezione di diverse cellule endocrine e neuroendocrine insieme ad altre sostanze e viene rilasciata insieme ad esse per esocitosi. Sia all'interno dei granuli o nel fluido extracellulare o nella circolazione, la CGA viene tagliata da enzimi tessuto-specifici in peptidi più piccoli che possiedono svariate attività biologiche. Alti livelli plasmatici di CGA sono stati correlati a tumori endocrini e neuroendocrini, ad alterazioni renali e cardiache ed è stato recentemente dimostrato cha la CGA viene prodotto non solo dalle cellule del sistema neuroendocrino diffuso ma anche da cellule di diversa derivazione embriologica. Inoltre, vengono scoperti ogni giorno nuovi effetti di peptidi derivati dalla CGA.
I principali peptidi derivati dalla CGA, correlati con la biologia del cuore, sono la Pancreastatina (PST) e le Vasostatine (VSs). La PST è un peptide di 49 aa, corrispondente alla CGA248-293 bovina e alla CGA240-288 umana, capace di stimolare la secrezione nelle cellule striali di ratto neonato. La Vasostatina I (VS-I) e la Vasostatina II (VS-II) corrispondono ai frammenti N-terminali CGA1-76 e CGA1-113 rispettivamente, e sembrano essere i migliori peptidi cardioregolatori nei mammiferi, dal momento che sono in grado di contrastare

l'inotropismo positivo mediato dai recettori b-adrenergici nei cuori dei vertebrati. Queste scoperte ci lasciano supporre che la CGA e i peptidi da essa derivati possano svolgere un ruolo regolatore nella secrezione cardiaca.
Questa "review" rappresenta un riassunto delle possibili relazioni esistenti tra la CGA ed i suoi peptidi, e la fisiologia, la biologia e la patologia del cuore.

## Introduction

Chromogranin A (CGA) is an acidic secretory protein detected in several endocrine and neuroendocrine tissues, co-stored and co-secreted with catecholamines. CGA undergoes tissue-specific proteolytic events, and it gives rise to several derived peptides with a wide range of functions and tissue targets, acting in an autocrine, paracrine and endocrine manner.

High levels of CGA have been detected, also, in pathological conditions regarding the neuroendocrine, the renal and the cardiac system, postulating its possible involvement in the patho-physiology of these diseases.

The purpose of this mini-review is to correlate the old and new knowledge about CGA and its derived-peptides with heart biology and physio-pathology, suggesting a modulation and cardiac-protective role for these molecules.

## CGA and CGA derived peptides: structural and functional features

CGA is a 48 kDa acidic water-soluble protein, identified for the first time by K. Helle in 1966 in the secretory granules of bovine adrenal medullary chromaffin cells, and successively detected also in other endocrine and nervous tissues. It represents the 40% of the soluble proteins of the granules, is co-stored with other granule secretory molecules such as catecholamines, enzymes, neurotransmitters, calcium, and is co-released by exocytosis upon splancnic nerve acetylcholine stimulation. Only after twenty years from its discovery, two distinct groups obtained for the first time the primary sequence of bovine CGA (see Figure1) [3, 11]. The analysis of the primary sequence shows many important features: a signal sequence, composed of the first 18 amino acid residues of the primary pro-peptide transcript, targeting it to rough endoplasmic reticulum membrane for maturation; numerous polyglutamic acid clusters in several regions of the molecule conferring a net negative charge, and an isoelectric point at about 5; two cysteine residues at positions 17 and 38 forming a disulphide bridge important for many CGA activities; an RGD sequences at the residues 43-45, probably implicated in adhesive processes between the cells and the extracellular matrix; several sites of post-translational modifications, such as O-glicosilation, phosphorilation and sulphation; twelve cleavage sites recognized by endopeptidase enzymes, five of them situated in the N-terminus domain and the others in the C-terminus.

CGA seems to have a double function: intracellulary, it binds both calcium and cate-

cholamines and it regulates the granule biogenesis acting as an "on/off switch"; extracellulary, it acts as pro-hormone. CGA is important as a whole molecule, but now they are becoming very important the several smaller peptides derived from this secretory protein, which showed to have different autocrine, paracrine or endocrine biological functions. In fact, CGA can be degraded by specific proteases (such as Prohormone convertases) either inside the granules, outsides in the extracellular environment or in the blood stream. This process and the fragments derived have a tissue- and specie-specific pattern. The peptides derived from the cleavage of bovine CGA and their functions are listed in Table 1.

**Vasostatins correlation with the heart: old and new findings**

CGA and its derived fragments have been detected also in the cardiac tissue. Recently, Glattard and colleagues [8] investigated the pattern of N-terminal CGA derived peptides in the cardiac tissue, revealing the presence of several VS-I- and VS-II-containing CGA fragments.

Vasostatin-I (VS-I) and Vasostatin-II (VS-II) derive from the cleavage at the first two pair of basic amino acids of the N-terminal domain of CGA, respectively K77-K78 and K114-R115 [15], giving rise to two peptides corresponding to the fragments $CGA_{1-76}$ and $CGA_{1-113}$ [7].

The prediction of the secondary structure of VS-I, the most abundant natural N-terminal CGA fragment, is compatible with an $\alpha$-helical orientation and shows three anphipatic domains: two in the N-terminal region, including the fragment $CGA_{1-16}$ and the fragment $CGA_{17-38}$ containing the cysteine-bridged loop, and the third in the C-terminus comprehending the fragment $CGA_{47-66}$. Moreover, the latest was well characterized and named chromofungin for its capability to cross the fungi membranes blocking their growing.

These fragments were named "Vasostatins (VSs)" because of their first role discovered: an inhibition of the vasoconstriction, evoked by the potent vasoconstrictor ET-1, on isolated segments of human internal thoracic artery and saphenous vein [1].

Subsequent evidences suggested a regulatory role for VSs acting via autocrine, paracrine or endocrine mechanisms in function of the cell/tissue target and the local peptide concentration [10]. VSs, in fact, promote the adhesion of fibroblasts and smooth muscle cells on extracellular matrix, exert autocrine inhibition of parathyroid hormone secretion in parathyroid cells, have an antimicrobial activity and, finally, have an effect on heart [20].

It has been well established that VSs exert a general negative effect on the physiology of eel, frog and rat hearts [2,6]. VSs and several other smaller CGA-N-terminal fragments (i.e.$CGA_{7-57}$, $CGA_{1-40}$, $CGA_{4-16}$ $CGA_{47-66}$), in fact, have been tested on isolated and perfused working hearts both in basal and isoproterenol (ISO)-stimulated conditions mimicking a $\beta$-adrenergic response. They acted modulating negatively the hearts inotropism on basal condition and counteracting the positive inotropism stimulated by the $\beta$-adrenergic agonist

ISO. This effect seems to be due to the fragment containing the disulfide-bridge loop, because the N- terminal fragments $CGA_{4-16}$ and $CGA_{47-66}$ are able to counteract the ISO-stimulated inotropy only at higher concentration than the peptide $CGA_{1-40}$ with the intact loop [19]. Moreover, VS-II shows a lower inotropic potency than VS-I, probably because of a different steric conformation. Both in eel, frog and rat hearts VS cardio suppressive effect depends on extracellular calcium influx, as previously showed by Aardal and Helle [1] in saphenous vein, and potassium channels. Furthermore, only in eel heart the intact endothelium is fundamental for VS activities. In fact, while in frog heart a treatment with Triton X-100 has no effect on VS activities, in eel heart it abolishes the VS-mediated inotropic effect.

Moreover, recent studies clarified the role of the cytoskeleton in VS signal, both in eel and frog hearts, being involved in several cellular functions, such as, for instance, the modulation of some ion channels (i.e. Calcium channels or Sodium channels) [14]. Using specific cytoskeleton inhibitors in concentrations which did not affect the functional integrity of the contractile cardiac machinery, it has been demonstrated a total block of the VS negative inotropism. Thus the cytoskeleton integrity plays a crucial role in the VS action.

Nevertheless, VS interaction with the cells has not been discovered yet and the pathway or pathways affected by VSs have not been well characterised yet, neither in mammals nor in other vertebrates. The most recent studies, in fact, deal with the insight of the possible cell-interaction way and with the characterization of a precise cellular pathway triggered or affected by VSs.

A classical and specific receptor for VSs has not been discovered yet, thus two possible different hypotheses of interaction with the cells could be taken into account [20]. The extracellular hypothesis proposes an interaction either with the membrane phospholipids or with some membrane proteins, for instance integrins, and a subsequent interference with the function of cellular effectors (I.E ion channels, receptors, enzymes). Blois and colleagues using a Langmuir film balance apparatus at 37°C demonstrated, in vitro, an interaction between VS-I at low concentration with membrane phospholipids in physiological conditions. The electrostatic and hydrophobic interaction affected the film fluidity [4]. On the other hand, we studied the effects of VS-I on 3D-cultured primary cardiomyocytes establishing its influence in extracellular matrix-cell interactions affecting the localisation of intracellular proteins such as endothelial nitric oxide synthase (eNOS) and HSP 90 [20]. The intracellular hypothesis suggests an internalisation of VS-I and its action inside the cell, as it has been demonstrated using rhodamine-labelled $CGA_{47-70}$ on smooth muscle layer of rat posterior cerebral artery [13]. Nevertheless, further studies have to be performed to clarify which hypothesis is correct, or whether both could be considered as combined interaction ways.

Another aim of the last studies was to detect the pathways involved in VS action.

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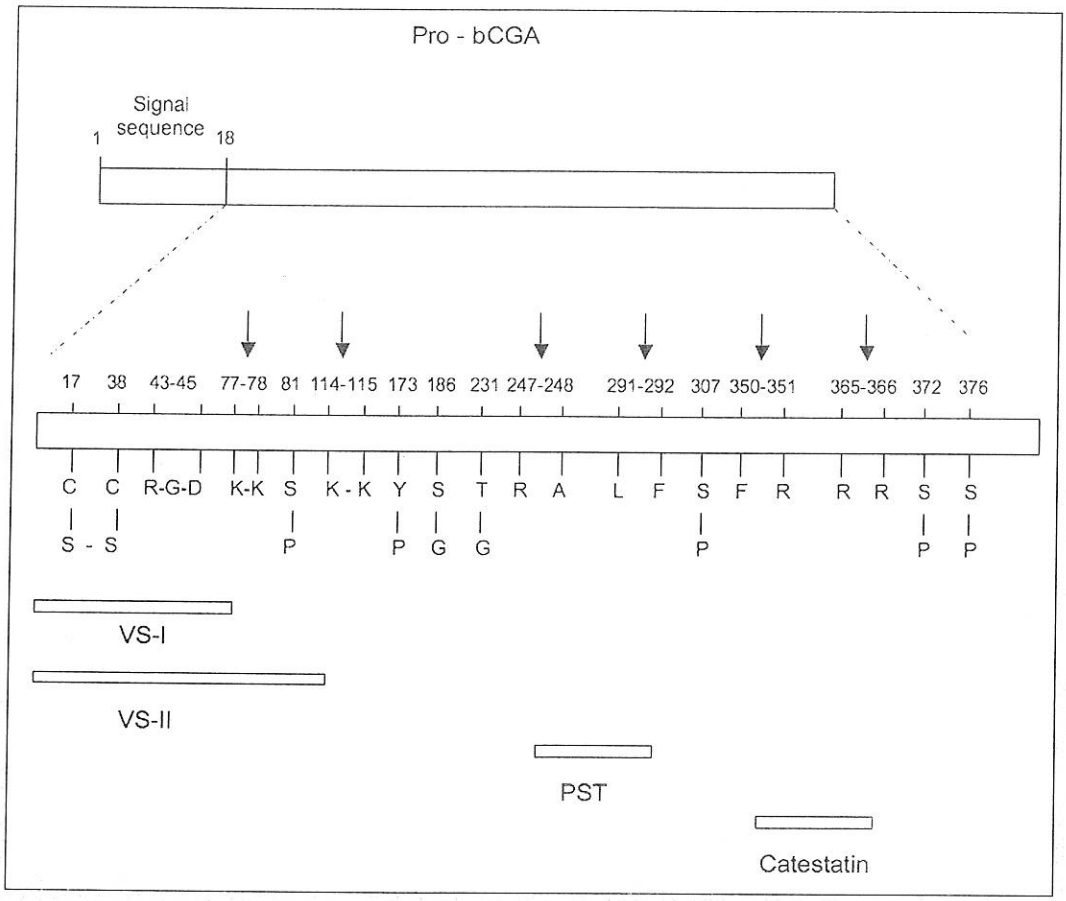



Figure 1. Schematic rapresentation of the main features of bovine CGA structure. (S-S) Disulphide bridge; (P) Phosphorylation; (G) Glycosilation; (▼) Cleavage site.

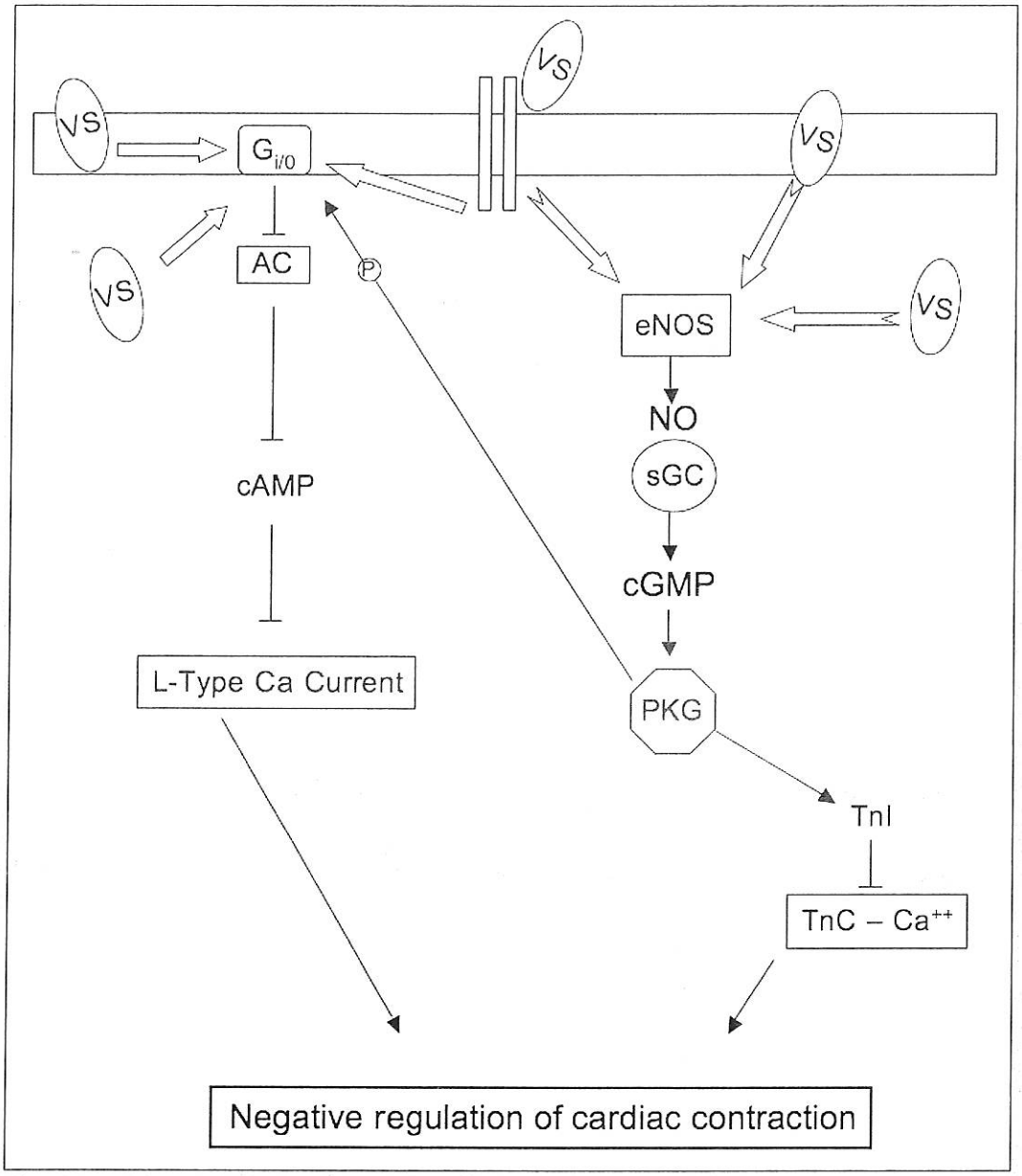
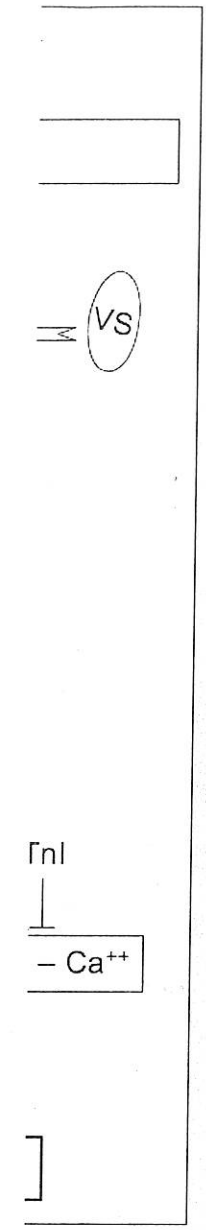



Figure 2. Hypothetical sites of action and pathways involving Vasostatin-I. (AC) Adenylyn Cyclate; (TnI) Troponin I; (TnC) Troponin C.

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Previously works carried out in eel and frog heart preparations, showed important differences in the pathways affected by VS-I, due to species-specific features. The implication of endocardial endothelium (EE) and the Nitric Oxide (NO)-cGMP signal transduction pathway seems to be essential in mediating VS effects only in eel heart [6, 18]. In fact, it has been demonstrated that the use of either NOS or soluble guanylate cyclase (GC) or cGMP-activated protein kinase (PKG) (an important NO target) inhibitors abolishes completely the effects of VS-I. The negative VS-inotropism can be explained considering that the NOS-dependent NO production increases the level of cGMP which activates PKG. PKG, on one hand, phosphorilates the troponin I and causes the decrease of the affinity of troponin C for calcium, regulating negatively the cardiac contraction; on the other hand, it phosphorilates the alpha-subunit of the $G_{i/0}$ protein, which negatively affects adenylyn cyclase, decreases the cAMP levels and finally inhibits the L-type Ca current. Lastly, both pathways elicit the negative regulation of the cardiac contraction. Recently, Cappello and colleagues [16] studied, for the first time, the pathways affected by VS-I in mammalian heart, using as a model the rat heart. Using specific inhibitors, they demonstrated the involvements of the same NOS-NO-cGMP-PKG system as in the eel heart.

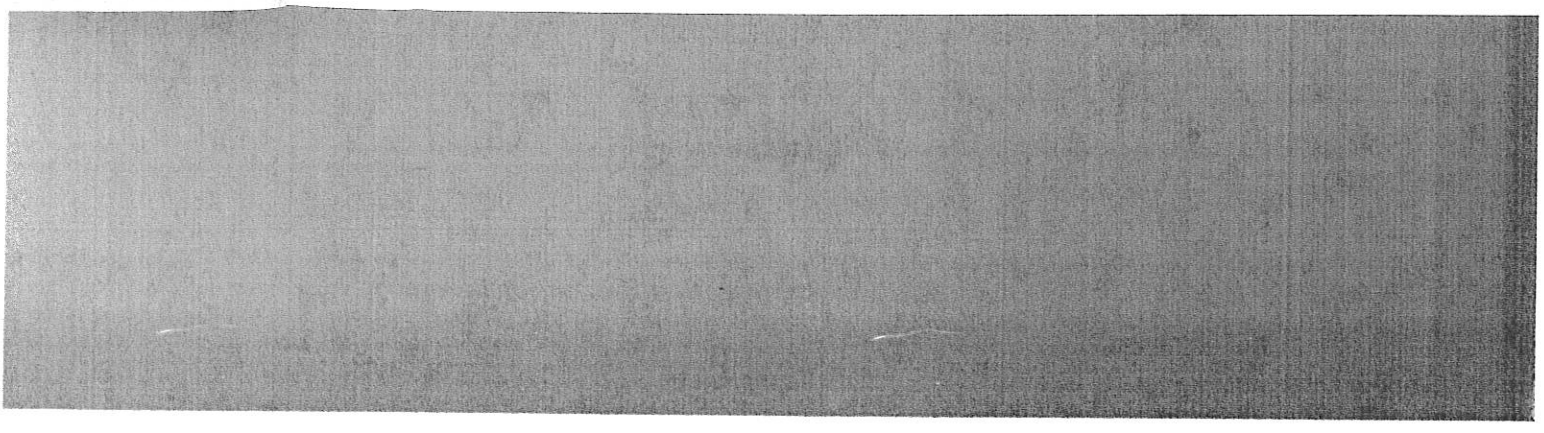
Gallo and co-workers [12] detected whether anti-adrenergic effects of VS-I occurs directly on cardiomyocytes or on other cell types of the cardiac tissue. They demonstrated that the synthesis of NO induced by VS-I takes places not in cardiomyocytes but in EE and it depends on Phosphoinositide-3 kinase (PI3K) activation.

**Role of CGA and CGA derived peptides in heart physio-pathology**

High serum levels of CGA have been correlated to several kind of neuroendocrine tumours for long time [9]. Recently high levels of CGA have been also reported in patients with heart failure and myocardial infarction [5]. Ceconi and co-workers correlated the increase of serum CGA with the chronic heart failure (CHF) [5], which is a syndrome characterized by neuroendocrine activation, in which the serum levels of catecholamines, natriuretic peptides and several peptides signalling system increase. These work demonstrated for the first time that serum CGA levels increased in patients with CHF and it was related to the clinical severity of the syndrome. The increase in CGA was found already in the early stages of the failure and it was a predictive factor of mortality. Moreover, it has been speculated that the high CGA levels may represent the response of the organism to counteract the effects of an excessive neuroendocrine activation. On the other hand, being CGA a component of the neuroendocrine system, the increase of its levels could be just the consequence of the over stimulation of the neuroendocrine system [5].

It has been well established that CGA either into the neuroendocrine vescicles or in the blood stream undergoes proteolytic events, giving rise to several CGA derived peptides





showing autocrine and paracrine effects. Moreover, it has been demonstrated that the cardiac tissue, in both in normal and pathological condition, is able to produce CGA [17]. Thus, CGA and its derived peptides could play an auto-endocrine regulation on the heart, counteracting an excessive adrenergic stimulation in normal condition and especially in pathological condition correlated with their high plasma levels. In this view CGA shows a role in myocardial protection. Recently, it has been, in fact, proposed a preconditioning-like effect of VS-I, if given before ischemia/reperfusion and it has been demonstrated a reduction of the infarct size after the administration of VS-I before I/R. Furthermore, it has been established the involvement of, at least, two different pathways: the NO-cGMP-PKG pathway and the adenosine-pathway. Indeed, the inhibition of eNOS blocked totally this effect and on the other hand the inhibition of A1-receptors reduced it about 50% [16].

Little is still known about the role of CGA and its derived peptides in physio-pathology of the heart and further studies are necessary. However, these molecules, acting as neurormones, could play a central role in the cardiac physio-pathology and could represent the main target of the new pharmacological therapy.

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**List of abbreviations**

| | | |
|---|---|---|
| CGA | = | Chromogranin A |
| VS-I | = | Vasostatin I |
| VS-II | = | Vasostatin II |
| PST | = | Pancreastatin |
| TNFα | = | Tumor necrosis factor α |
| ET-1 | = | Endothelin-1 |
| ANF | = | Atrial Natriuretic Factor |
| PLC | = | Phospholipase C |
| IP3 | = | Inositol 1,4,5 triphosphate |
| DAG | = | Diacylglycerol |
| PKC | = | Protein Kinase C |
| ISO | = | Isoproterenol |
| eNOS | = | Endothelial nitric oxide synthase |
| EE | = | Endocardial endothelium |
| NO | = | Nitric oxide |
| GC | = | Guanylate cyclase |

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PKG = cGMP-activated protein kinase
PI3K = Phosphoinositide-3 kinase
CHF = Chronic Heart Failure

Table I. Bovine CGA derived peptides and their main function

| Peptides | Function |
|---|---|
| Vasostatin I ($CGA_{1-76}$)<br>Vasostatin II ($CGA_{1-113}$)<br>(rat-βgranin ($CGA_{1-128}$)) | Inhibition vasoconstriction |
| | Promotion of adhesion |
| | Inhibition parathyroid hormone secretion |
| | Antimicrobial effects |
| | Negative inotropism |
| Chromofungin ($CGA_{47-66}$) | Antimicrobial effects |
| Chromostatin ($CGA_{124-143}$) | Inhibition chromaffin cells secretion |
| Chromacin ($CGA_{173-194}$) | Antimicrobial effects |
| Pancreastatin ($CGA_{248-293}$) | Inhibition of glucose-stimulated insulin secretion<br>Take part in lipid and glucose metabolism |
| WE-14 ($CGA_{316-331}$) | No Data |
| Parastatin ($CGA_{347-419}$) | Inhibition of parathormone and CGA secretion from parathyroid cells |
| Catestatin ($CGA_{344-364}$) | Inhibition of catecholamine secretion; promotion histamine release in mast cells |

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