Glycosylated Chromogranin A:

Potential Role in the Pathogenesis of Heart Failure

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ABSTRACT

Purpose of review: Endocrine and paracrine factors influence the cardiovascular system and the heart by a number of different mechanisms. The chromogranin-secretogranin (granin) proteins seem to represent a new family of proteins that exerts both direct and indirect effects on cardiac and vascular function. The granin proteins are produced in multiple tissues, including cardiac cells, and circulating granin protein concentrations provide incremental prognostic information to established risk indices in patients with myocardial dysfunction. In this review, we provide recent data for the granin proteins in relation with cardiovascular disease, and with a special focus on chromogranin A and heart failure.

Recent findings: Chromogranin A is the most studied member of the granin protein family and shorter, functionally active peptide fragments of chromogranin A exert protective effects on myocardial cell death, ischemia-reperfusion injury, and cardiomyocyte Ca^{2+} handling. Granin peptides have also been found to induce angiogenesis and vasculogenesis. Protein glycosylation is an important post-translational regulatory mechanism and we recently found chromogranin A molecules to be hyperglycosylated in the failing myocardium. Chromogranin A hyperglycosylation impaired processing of full-length chromogranin A molecules into physiologically active chromogranin A peptides and patients with acute heart failure and low rate of chromogranin A processing had increased mortality compared to other acute heart failure patients. Other studies have also demonstrated that circulating granin protein concentrations increase in parallel with heart failure disease stage.

Summary: The granin protein family seems to influence heart failure pathophysiology and chromogranin A hyperglycosylation could directly be implicated in heart failure disease progression.

INTRODUCTION

Endocrine and paracrine systems in heart failure

Endocrine and paracrine factors influence the cardiovascular system and the heart by a number of different mechanisms. The importance of hormonal substances for heart failure (HF) progression is demonstrated in today's HF therapies that include β -adrenergic blockers, angiotensin converting enzyme inhibitors, angiotensin II receptor blockers, aldosterone inhibitors, and most recently the angiotensin receptor-neprilysin inhibitor Entresto (Novartis, Basel, Switzerland) [1]. These drugs all influence peptide hormonal systems and reduce morbidity and mortality in patients with HF with reduced ejection fraction. Substantial experimental data also suggest that excessive β -adrenergic activity and enhanced tone in the renin-angiotensin-aldosterone axis are key factors for left ventricular (LV) remodeling and HF development [2]. In contrast, natriuretic peptides and some other paracrine and endocrine substances have protective properties that counteract some, but not all of the detrimental effects from mechanical and hormonal stress pathways on the failing myocardium [3]. Of special relevance here is dysfunctional cardiomyocyte Ca^{2+} handling, which is a hallmark of HF development and directly related to LV systolic and diastolic function and the risk of ventricular arrhythmias [2]. As cardiomyocyte Ca^{2+} handling is not closely regulated by the natriuretic peptides, it is plausible that additional endocrine and paracrine protein protective systems could operate to counteract pathological cardiomyocyte Ca^{2+} handling in HF. Myocardial ischemia is also important for disease progression in patients with coronary artery disease and protein systems that enhance myocardial angiogenesis and vasculogenesis will counteract HF development in these subjects. Accordingly, studies that explore and characterize additional endocrine and paracrine protein systems have the potential to improve our understanding of HF progression. Still, such studies can be complex as most protective substances are processed from larger pro-hormones, and post-translational glycosylation can

impact processing from pro-hormones to shorter, physiologically active peptide fragments. This has recently been demonstrated for processing from pro-B-type natriuretic peptide (proBNP₁₋₁₀₈) to physiologically active BNP₁₋₃₂ [4], and post-translational regulation of proteins via myocardial hyperglycosylation seems to be a universal mechanism in the failing myocardium [5].

The close correlation between clinical outcomes and circulating concentrations of endocrine and paracrine substances, which are produced in the body as a compensatory mechanism in parallel with disease progression, also makes these proteins excellent HF biomarkers. This is true for BNP and N-terminal proBNP (NT-proBNP), and these biomarkers are included in guidelines for early diagnosis of acute and chronic HF [1]. Furthermore, additional cardiac biomarkers like ST2 and growth differential factor-15 also seem to protect the myocardium by controlling inflammation after cardiac injury [6, 7]. Thus; studies of novel endocrine and paracrine protein systems can improve HF management by providing knowledge of HF pathophysiology, new therapeutic strategies, and new biomarker candidates. The chromogranin-secreto granin (granin) proteins could represent one such new family of proteins that has direct and indirect effects on cardiac and vascular function and may also serve as novel prognostic biomarkers during myocardial dysfunction [8]. The granin proteins are produced in multiple tissues, including in cardiac cells and can thus be classified both as endocrine and paracrine substances. In this review we will present and discuss the most recent results related to the granin proteins in cardiovascular disease with a special focus on chromogranin A and HF development.

CHROMOGRANIN-SECRETAGRANIN (GRANIN) PROTEIN FAMILY

Characteristics of the granin proteins

Chromogranin A (CgA), together with chromogranin B (CgB) and secretogranin II (SgII) constitute the main members of the granin protein family [9]. The granin proteins are characterized by a high proportion of acidic amino acids and the ability to bind Ca^{2+} [8]. CgA, CgB, and SgII also contain numerous dibasic cleavage sites along the amino acid structures of the molecules [8]. Accordingly, the granin proteins have several characteristics in common with other prohormones, but can be differentiated from these other prohormones by the large size of the granin proteins (>400 amino acids for CgA, CgB, and SgII). The granin proteins also differ from other prohormones during processing as most prohormones are only processed into two peptide fragments (e.g. BNP and NT-proBNP), while 9-16 cleavage sites have been reported for CgA, CgB and SgII [8]. Processing of granin proteins are also more complex than for most other prohormones with cleavage leading to peptide fragments of variable length, including truncated peptides [8, 10]. The principal proteases that cleave granin proteins are proconvertase (PC) 1/3 and PC2 [8], but also additional proteases can process granin proteins, including furin [11], the fibrinolytic enzyme plasmin [12, 13], and the cysteine protease cathepsin L [14].

Regulation of granin protein production during heart failure development

CgA is the principal member of the granin protein family and the member that has been studied most extensively. CgA was first described in the adrenal medulla in 1966 [15] and has later been found to have a widespread production in endocrine and neuronal tissue [16-18]. Due to the widespread production of CgA in neuroendocrine tissues, CgA is currently in clinical use as a biomarker of neuroendocrine tumors, such as carcinoids and pheochromocytomas [17]. More recently, CgA production has been reported also in non-

neuroendocrine cells, including production in cardiac cells in situations of myocardial dysfunction [19-20] and CgA concentrations are increased in patients with HF [21]. CgB and SgII production is also prominent in neuroendocrine cells, but the ratio between CgA: CgB: SgII seems to differ according to the organ being studied. For example, CgA is produced in excess of the other granin proteins in the adrenal medulla, while SgII production seems relatively high in the posterior pituitary [22, 23]. Recent publications have also found CgB and SgII production in cardiac cells and the proteases that cleave the granin proteins are also increased in the failing myocardium [10, 24]. Accordingly; both granin protein production and processing appear to be markedly enhanced in the myocardium of subjects with HF, which suggests that these proteins could play a role as endocrine and paracrine substances in HF pathophysiology. In general, a common feature of cells producing chromogranin-secretogranins appears to be marked secretion of proteins or other substances, which is a phenotype also characterizing tumor cells [25-28] and cardiomyocytes during heart failure development [19]. It is also possible that a dysregulated intracellular Ca^{2+} homeostasis could trigger granin production as this has been demonstrated for granin proteins in neuroendocrine cells [29, 30], but it is not known whether this mechanism regulates granin gene expression also in non-neuroendocrine cells. Excitation-transcription coupling has also been established as a key mechanism for increased granin protein expression in non-cardiac cells; i.e. transmission of action potentials to neuroendocrine cells will lead to a direct increase in chromogranin-secretogranin gene expression via specific promotor regions in *CHGA*, *CHGB*, and *Scg2* (gene names for CgA, CgB, and SgII) [31]. Currently, no information is available whether mechanisms analogous to excitation-transcription coupling could increase granin protein expression in cardiac cells.

Physiological properties of the full-length granin proteins in cardiovascular disease

Physiological properties of the granin proteins can be separated according to functions related to the full-length pro-proteins and actions of shorter peptide fragments derived from CgA, CgB, and SgII after intra- or extracellular processing (Table). The ability of CgA, CgB, and SgII to function as high-capacity, low-affinity Ca^{2+} binding proteins is important for granulogenesis in cells that secrete hormones and other substances. In these cells, granin proteins bind Ca^{2+} at the center of the granules, thereby stabilizing these intracellular vesicles to permit transport of proteins and other substances from the Golgi apparatus to the cell membrane [8]. Experimental studies manipulating intracellular expression of CgA, CgB, and SgII have all found marked disruption in intracellular trafficking, which support a crucial role of the granin proteins for cellular release of hormones and paracrine substances from various cell types. More recently, manipulation of intracellular CgB content in cardiomyocytes was also found to affect proBNP production [24]. Thus, the granin proteins seem directly implicated in natriuretic peptide production and release during HF development. Whether alterations in intracellular CgA content may influence proBNP production is not known, but it is possible as previous studies have found CgA and CgB to be co-localized with natriuretic peptides in cardiomyocytes [32] and there is redundancy for functional properties between the granin proteins.

The full-length granin pro-proteins also regulate intracellular Ca^{2+} homeostasis across different cell types by modulating opening probability of the inositol 1,4,5-triphosphate receptor/ Ca^{2+} channel (IP3R) [33], which is considered a Ca^{2+} channel of increasing importance also in the myocardium [34, 35]. Of note; modulation of IP3R activity was postulated as the mechanism whereby CgB modulates proBNP production in cardiomyocytes [24]. It is also possible that short fragments of the pro-proteins could regulate IP3R as only

shorter sections of the pro-proteins interact with the receptor [36], but this has not been studied in detail. The close association between granin proteins and intracellular Ca^{2+} fluxes is also reflected in excitation-transcription coupling for granin production [31]. Accordingly; data from different types of tissue suggest that the chromogranin-secretogranin protein family should be considered an endocrine and paracrine protein system that has intracellular Ca^{2+} handling as one of its primary objectives. The relevance of this protein system for cardiovascular disease and HF progression has been validated in mice lacking the gene for CgA (*CHGA*^{-/-}), which resulted in disruption of intracellular protein transport in endocrine organs with alterations in several hormonal systems [37]. The *CHGA*^{-/-} mice also exhibited clear evidence of structural heart disease with increments in LV mass and also other echocardiographic evidence of Stage B HF.

Physiological properties of granin peptide fragments in cardiovascular disease

Although the full-length pro-hormones seem to have important roles for stabilizing intracellular protein transport and for regulation of IP3R activity, functional properties of the granin proteins is also closely associated with the production of several short peptide fragments [8]. Proteolytic processing of especially CgA and SgII generates several peptides that have been found to have functional properties across a wide range of organs and tissues. Of relevance for cardiovascular disease and HF development, these granin peptide fragments seem to have both direct and indirect effects on the myocardium (Table). Of note; infusion of the CgA fragment catestatin (CST, CgA₃₅₂₋₃₇₂) in *CHGA*^{-/-} mice restored circulating catecholamine concentrations [37], possibly through non-competitive binding to the muscarinic receptor in the adrenal medulla, and inhibited LV remodeling [37]. CST is a highly conserved CgA fragment that was first identified as a potent inhibitor of catecholamine release from the adrenal medulla [38]. CST appears to be one of the most important granin

peptide fragments for HF development as CST also previously has been found to counteract excessive β -adrenergic and endothelin-1 signaling in the myocardium [39]. CST has also been found to reduce ischemic-reperfusion injury and cellular apoptosis after vascular injury [40] and to reduce arterial hypertension [41-44]; thus, CST seems to counteract stress on the myocardium by several mechanisms. CST also seems to play a role in innate immunity as CST can induce migration of monocytes and regulate Ca^{2+} entry via directly calmodulin-binding in neutrophil cells [45, 46]. Other granin peptide fragments like vasostatin I from CgA (CgA₁₋₇₆) and secretoneurin from SgII (SN, (SgII₁₅₄₋₁₈₆)) also have cardioprotective properties, including the ability to reduce ischemic-reperfusion injury and cellular apoptosis [10]. The actions of the peptide fragments therefore suggest that the granin protein family should be considered an endocrine and paracrine system of importance for the protection from ischemic vascular injury. Vasostatin has also been found to dilate arterial and venous blood vessels, which will reduce blood pressure [47]. In addition, recent data for granin peptide fragments supports the importance of the granin protein family for intracellular Ca^{2+} handling, including in cardiomyocytes. We recently demonstrated that SN from SgII could directly bind to and inhibit calmodulin (CaM) and Ca^{2+} /CaM-dependent protein kinase II δ (CaMKII δ) [48], which are two kinases in the same pathway and implicated in driving HF progression [49]. Of note, inhibition of overactive CaMKII δ activity is currently considered one of the most attractive targets for development of new HF therapy [2]. CST has also been linked to cardiomyocyte Ca^{2+} dysfunction, although previously only through indirect mechanism involving endothelial cells and production of NO [39, 50]. We have now also demonstrated direct affects by CST on cardiomyocyte Ca^{2+} handling via direct CaMKII δ inhibition [51], thus linking CST directly to the regulation of cardiomyocyte Ca^{2+} handling. Accordingly; taken together, the chromogranin-secretogranin protein family appears to represent an endocrine and paracrine protein systems that regulates intracellular Ca^{2+}

handling, and therefore could play an important role in the myocardium during HF progression.

The granin proteins are prognostic biomarkers in patients with myocardial dysfunction

Given the putative important pathophysiological properties of the granin proteins in relation to HF progression, it is not surprising that circulating concentrations of the granin proteins could have prognostic utility in subjects with myocardial dysfunction. Previous studies have demonstrated that myocardial and circulating granin concentrations are increased in parallel with HF severity [10, 21, 52, 53], although the diagnostic utility of granin proteins to diagnose HF is inferior to the accuracy of BNP and NT-proBNP. The first study to report increased circulating CgA concentrations in patients with HF was published in 2002 and these authors also reported that CgA measurements provided additional prognostic information to established risk indices, including BNP concentrations [53]. Subsequent studies demonstrated independent prognostic information from CgA measurements also in subjects with acute myocardial infarction [54, 55]. We then measured CgA in 1268 patients with acute coronary syndromes and found admission CgA concentrations to provide additional prognostic information across the different categories of patients, including in subgroups with BNP measurements and estimation of LV ejection fraction [56]. In contrast, we did not find CgA concentrations to add to established risk indices in 1233 patients included in the multicenter, randomized, prospective, double-blind GISSI-HF trial of patients with chronic HF [21], suggesting that CgA measurements may have most clinical potential in patients with acute cardiovascular disease. We and other groups have later also demonstrated that CgA concentrations provide additional prognostic information to established risk indices in patients with acute respiratory failure and in patients with severe sepsis and septic shock [57, 58].

Biomarker studies of granin peptide fragments and implications for granin processing

More recently, studies have also started to explore whether assays that only measure specific fragments of CgA will provide prognostic information. However, contrary to the situation for full-length CgA, these studies have not found positive correlations between concentrations of these fragments and disease progression in cardiovascular disease. For example, one Chinese study found low circulating vasostatin-II concentrations to be associated with disease severity in patients with coronary artery disease [59] and another study reported that subjects with subclinical and established HF had reduced circulating CST levels compared to subjects free from HF [60]. Patients with hypertension, which is a risk factor for HF development, have reduced circulating CST concentrations compared to normotensive subjects [41]. Still, it should be acknowledged that there is some heterogeneity in published results for CgA fragments and this could partly relate to the use of assays that also cross-react with the full-length CgA protein [61]. Still, the positive correlations between full-length CgA concentrations and disease progression, but no such correlations for CgA fragments and disease progression, could indicate that processing of the pro-hormone to functionally active, shorter CgA peptides is impaired in patients with myocardial dysfunction. In contrast, processing appears more limited for CgB, where we also have reported increased circulating CgB concentrations in parallel to HF disease progression [52]. We also found CgB to provide prognostic information in subjects with acute respiratory failure [62]. SgII also seems to be fully processed to SN with SgII immunoreactivity in plasma primarily in the form of free SN [63]. We have also validated the presence of free SN in the circulation of patients with HF by liquid chromatography mass spectrometry [48]. Moreover, using an in-house radioimmunoassay, we and collaborators have found circulating SN concentrations to add to established risk indices in patients with acute HF [48], patients with ventricular arrhythmia-induced cardiac arrest [48], patients with severe sepsis and septic shock [64], and in patients

with cardiovascular related-acute respiratory failure [65]. Other groups have also demonstrated prognostic value of circulating SN concentrations after cardiac arrest [66].

Cardiovascular pathophysiology associated with high circulating granin concentrations

Although the granin proteins have similar functional properties, there are distinct differences between these proteins that could impact also the potential of these proteins as cardiovascular biomarkers. For example; while circulating CgA and CgB concentrations are easily influenced by physical activity, SN concentrations only rise after prolonged strenuous physical activity [67]. This could impact also the prognostic potential of these proteins as CgA and CgB concentrations more closely will reflect sympathoadrenergic tone and thus have potential regardless of disease etiology, whereas SN measurements may relate more directly to specific cardiovascular pathophysiology. This is also in line with data so far where CgB provided prognostic information regardless of etiology for acute respiratory failure [62], while SN concentrations only predicted outcome in the patients with cardiovascular related-acute respiratory failure in the same study [65]. Of note, we found circulating SN concentrations to provide stronger prognostic information than CgA concentrations in patients with acute HF [48] and in patients with severe sepsis and septic shock [64], which supports the potential of SN as a novel prognostic biomarker in subjects with myocardial dysfunction.

Circulating concentrations of CgA, CgB, and SN are only moderately correlated ($\rho \approx 0.5$), and these peptides are also weakly to moderately correlated with established biomarkers like BNP/NT-proBNP and high-sensitivity troponin concentrations ($\rho \approx 0.3-0.4$) [21, 51, 57, 64, 67]. Several studies have also failed to demonstrate significant correlations between circulating granin concentrations and catecholamine concentrations [48, 54, 55, 67], but this could at least partly relate to analytical issues as catecholamine measurements require

stringent pre-analytical handling of blood samples and the half-lives of epinephrine and norepinephrine are very short. We have demonstrated a positive correlation between circulating SN concentrations and free cortisol concentrations in patients with severe sepsis and septic shock [64], thus measurements of granin protein concentration should be considered reflective of important endocrine stress pathways. Accordingly; circulating granin protein concentrations could integrate information on endocrine stress pathways and specific myocardial pathophysiology in patients with myocardial dysfunction, which could explain the strong and additional prognostic information by these biomarkers to established biomarkers and risk indices in patients with myocardial dysfunction [48, 51, 53-58, 62, 64-66]. Circulating concentrations of the granin proteins are also influenced by renal dysfunction and, although we have adjusted for indices of renal function in multivariate analyses in the different cohorts, this could also explain some of the prognostic value of these proteins in patients with cardiovascular disease. Regardless of mechanism, the strong prognostic value of especially CgA and SN across different cohorts of patients with myocardial dysfunction and cardiovascular disease supports the model of the granin protein family as a new endocrine and paracrine protein axis with relevance for cardiovascular disease. Moreover, given the multiple protective actions of the granin peptide fragments (Table), it is likely that impaired processing of the pro-hormones to functionally active, shorter peptide fragments would drive HF disease progression.

Myocardial chromogranin A glycosylation is increased in HF and impacts chromogranin A processing

Previous studies with assays measuring the full-length CgA protein have found CgA concentrations to increase in parallel with HF disease severity [21, 52, 53], while circulating CST concentrations were reduced in patients with stage B HF [60]. This could indicate

impaired processing of CgA to CST during HF disease progression. On gel electrophoresis in myocardial tissue samples from mice with HF, we had also noticed CgA bands with very high molecular mass, often 150-250 kDa [51], while the estimated molecular mass of full-length CgA is only 74 kDa [69]. We also saw a consistent reduction in shorter CgA fragments in HF individuals with high-molecular weight CgA bands [51], which strongly indicated that myocardial processing of the full-length CgA pro-protein to functionally active CgA fragments could be reduced during HF progression. Other groups had also published immunoblots from myocardial tissue with CgA immunobands >74 kDa [20, 70]. In contrast, we did not see any high-molecular weight CgA bands outside of myocardial tissue samples [51]. We therefore hypothesized that CgA either is post-translationally modified or bound to other CgA molecules (protein-protein-binding) in the failing myocardium. After performing experiments that excluded the possibility of protein-protein-binding, we started to explore the hypothesis that post-translational modifications could explain the myocardial high-molecular weight CgA bands in HF individuals. Based on the recognized importance of glycosylation for post-translational modification in HF [5], we first performed a series of experiments adding various cocktails of deglycosylation enzymes before immunoblotting. This yielded a significant downward shift in the high-molecular weight bands and increments in short CgA bands, which supported the model of excessive glycosylation being responsible for reduced CgA processing in the failing myocardium [51]. We also found that cocktails of deglycosylation enzymes that cleaved both O- and N-glycosylation sites were most efficient in reducing the high-molecular weight CgA bands in the myocardial tissue samples.

Chromogranin A hyperglycosylation could impact heart failure disease progression

The importance of glycosylation for post-translational regulation of CgA processing is consistent with the structure of CgA, which has been found to include three O-glycosylation

sites [68] and one N-glycosylation site [51] (Figure). O-linked glycosylation occurs when glycans are attached to serine or threonine residues, while N-linked glycosylation take place when glycans are attached to asparagine residues. Of note, glycosylation of proBNP has previously been shown to reduce processing to BNP and NT-proBNP by blocking of the access for the proteases [4, 71, 72]. Analogous to this, hyperglycosylation of CgA with subsequently reduced concentrations of CgA fragments like CST and vasostatin in the failing myocardium will likely contribute to HF disease progression given the important physiological properties of these peptide fragments (Table). Supporting this model, we also found that HF patients with low CgA:CST conversion had significantly higher mortality compared to HF patients with higher CgA:CST conversion [51]. Thus; both experimental and clinical data support that myocardial CgA hyperglycosylation will impact HF disease progression. Still, there are a number of unanswered questions, including the mechanism for the increased glycosylation of CgA in the failing myocardium. A previous study of HF patients using HPLC has also provided evidence of circulating CgA molecules with higher molecular mass compared to the estimated molecular mass for full-length CgA [19], but the functional consequences of this and possible implications for CgA as a biomarker in HF have not been established. Of note, glycosylation of biomarkers may impact the diagnostic performance of different assays as previously reported for the proBNP II assay for NT-proBNP measurements (Roche Diagnostics, Penzberg, Germany) [71]. CgA glycosylation could therefore explain some of the discrepant results between studies using different CgA assays. Finally, although CgA seems to be hyperglycosylated in the failing myocardium, we have not found indications of increased CgB or SgII glycosylation in HF subjects [10, 52]. As the granin proteins co-localize in myocardial tissue [32] and has similar physiological properties, it is possible that for example SN could compensate partly for reduced CST levels in the myocardium of HF individuals, at least related to local CaMKII δ control. Still, although

more studies are needed to further define the functional consequences of CgA hyperglycosylation in the failing myocardium, the last decade of experimental and clinical studies clearly indicate that the granin protein family should be included as an important endocrine-paracrine system in cardiovascular disease, and especially related to protection from vascular injury and regulation of intracellular Ca^{2+} homeostasis.

CONCLUSION

The granin protein family is an endocrine and paracrine protein system that seems to have functional properties of relevance for cardiovascular disease and potential as novel cardiovascular biomarkers. Myocardial CgA is glycosylated in HF individuals, which likely contributes to disease progression as a result of impaired processing of the full-length CgA molecule with subsequently reduced concentrations of shorter, functionally active CgA peptide fragments.

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- Of importance
- Of major importance

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Table. Overview of functional properties of the granin proteins in relation to cardiovascular disease

Full-length CgA, CgB, and SgII

CgA		
Year	Function	Localization
2005	Reduced number and size of chromaffin granules [37]	<i>CHGA</i> ^{-/-} mice
2007	Production [19]	Human heart
2013	Directly influenced myocardial and coronary function by Akt/nitric oxide synthase/nitric oxide/cGMP/protein kinase G pathway. The heart generates intracardiac CgA fragments in response to hemodynamic and excitatory challenges. [70]	Rat hearts
CgB		
2008	Regulator of cardiomyocyte InsP3/Ca ²⁺ -dependent signaling, nuclear factor κB activity, and BNP production. [24]	Rat cardiomyocytes
SgII		
2012	Increased production and processing in the left ventricle. [10]	Mouse model with myocardial infarction and heart failure

Peptide fragments

CHROMOGRANIN A (CgA)

CATESTATIN (CST, CgA₃₅₂₋₃₇₂)		
Year	Function	Localization
1998	Vasodepression activity by stimulating histamine release [73]	Rat model
2002	Plasma levels inversely correlate with hypertension [41]	Hypertensive patients
2005	Increased vasodilation [43]	Forearm blood flow in healthy individuals
2005	Restored circulating catecholamine concentrations and inhibited LV remodeling [37]	<i>CHGA</i> ^{-/-} mice
2007	The catestatin Gly364Ser variant reduced risk of developing hypertension, especially in men. [42]	Humans
2008	Induced migration of monocytes [45]	Human peripheral blood monocytes
2008	Inhibited of isoproterenol and endothelin signaling [74]	Frog heart
2008	Counteracted excessive β-adrenergic and endothelin-1 signaling, and the authors indicated involvement of β2-AR-Gi/o protein-NO-cGMP pathways [39]	Rat hearts
2009	Induced migration of monocytes and regulate Ca ²⁺ entry via directly calmodulin-binding [46]	Human Neutrophil cells
2009	Improved baroreflex sensitivity [75]	<i>CHGA</i> ^{-/-} mice
2010	Improved heart rate variability [76]	<i>CHGA</i> ^{-/-} mice

2010	Modulated myocardial function in fish [77]	Fish heart
2010	Attenuated I/R injury [78]	Rat heart
2010	Production of CST in cardiac cells [20]	Murine heart
2010	Reduced ischemic-reperfusion injury and cellular apoptosis after vascular injury and reduced arterial hypertension [40]	hindlimb ischemia model
2010	Increased vasodilation in vivo, especially in females [44]	Humans
2011	Anti-adrenergic action of CST relies on the endothelial PI3K-AKTeNOS pathway [79]	Rat papillary muscles and cardiomyocytes
2012	Induced negative inotropism and lusitropism by NO signaling [50]	Rat heart
2012	Improved Frank-Starling response [80]	Spontaneously hypertensive rats and normal rats
2013	Cardioprotection in early reperfusion stage involves mitoKATP channel, ROS signaling and prevention of mPTP opening through upstream PI3K/AKT and PKC signaling [81]	Rat hearts
2015	Increased cell survival rate and decreased cell contracture, and this relied on maintenance of mitochondrial membrane potential and increased phosphorylation of AKT [82]	Rat cardiomyocytes undergoing simulated I/R
2017	Myocardial CgA-to-CST conversion reduced because of hyperglycosylation [51]	Mouse hearts
2017	Improved cardiomyocyte Ca ²⁺ handling via CaMKII inhibition [51]	Mouse cardiomyocytes
2017	Reduced CgA levels and CgA-to-CST levels is associated with poor prognosis [51]	Heart failure patients
VASOSTATIN I and II (VS-I, CgA1-76; VS-II CgA1-113)		
1992	VS-I inhibited vascular contractility [47]	Human blood vessels
2002	Inhibited myocardial inotropy [83]	Frog heart
2003	VS-I and VS-II activated a calcium-dependent negative inotropism and counteracted the positive inotropism exerted by isoprenaline. The disulphide-bonded CgA17–38 was essential both effects. [84]	Frog heart
2006	Negative inotropism by VS-I without affecting coronary pressure	Rat heart
2007	VS-I protected against ischemia-induced myocardial necrosis, presumably involving the endothelial adenosine/nitric oxide signaling pathway. [85]	Rat heart
2012	Antiarrhythmic effects of VS-I. [86]	Canine model of atrial fibrillation
2012	Protected against hypoxia/reoxygenation injuries. [87]	Rat cardiomyocytes
2015	The C-terminal sequence CgA79-113 of VS-II inhibited the vaso-constrictive effects of angiotensin II in vitro and that this effect is mediated by the angiotensin II type 2 receptor. [88]	Rat kidney and human plasma
2016	VS-I treatment inhibited the progression of hypertrophy, fibrosis, and improved cardiac function. Ca ²⁺ handling was also improved, and the eNOS-cGMP-PKG pathway may mediate this. [89]	Rat model of ISO infusion
CHROMOFUNGIN (CgA CgA47-66)		
2015	Induced negative inotropic effects without changing coronary pressure, this involved the AKT/eNOS/cGMP/PKG pathway. Also, protected against I/R myocardial injuries, this involved PI3K, RISK pathway, MitoKATP and miRNA-2. [90]	
SERPININ (CgA403-428)		
2012	Produced in the heart and act as novel β -adrenergic-like cardiac modulators. [91]	Rat heart

SECRETAGRANIN II (SgII)

SECRETONEURIN

Year	Function	Localization
2009	Induced angiogenesis and vasculogenesis by a nitric oxide-dependent mechanism [92]	Mouse hindlimb ischemia model
2012	SN gene therapy improved cardiac function after myocardial infarction [93]	Rat MI model
2012	Protects from ischemia-reperfusion injury and cardiomyocyte apoptosis. [10]	Rat hearts and cardiomyocytes
2013	Improved blood flow in an ischemia model and reduces necrosis and apoptosis [94]	Diabetic mice
2015	SN gene therapy improved cardiac ischaemia [95]	Apo E-/- mice
2015	Internalization by endocytosis, and direct inhibition of CaMKII activity and improvement of calcium handling. [48]	Mouse Cardiac cells

Figure. Overview of CgA and the CgA-derived fragment CST. One disulfide bridge and three known O-glycosylation sites in the CgA molecule are indicated. **(B)** In heart failure hyperglycosylation of CgA impairs efficient processing of CgA to the small fragment CST.