Chromogranin A: a novel factor acting at the cross road between the neuroendocrine and the cardiovascular systems

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Chromogranin A (CHGA) is a secretory protein stored in and released from neurons and cells of the diffuse neuroendocrine system. Cells of the adrenal medulla and adrenergic terminals are a main source of CHGA but also myocardial cells produce it under stress conditions. After secretion, CHGA is cleaved into several biologically active fragments, including vasostatins and catestatin. CHGA and its proteolytic peptides exert a broad spectrum of activities on the cardiovascular system. They act on blood pressure by controlling the vascular tone and the cardiac inotropic and chronotropic function. CHGA revealed to be a sensitive marker of myocardial dysfunction, with a high predictive power of morbidity and mortality in heart failure and ischemic heart disease. In addition, CHGA has been involved in the control of sustained endothelial inflammation and has been shown to be a good marker of persistent vascular inflammation in rheumatologic disorders affecting vessels. *J Hypertens* **29:409–414 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

Journal of Hypertension 2011, **29**:409–414

Keywords: catecholamines, chromogranin A, heart failure, hypertension, vasculitis, vasostatin

Abbreviations: BNP, brain natriuretic peptide; cAMP, cyclic adenosine monophosphate; CHGA, chromogranin A; CRE, cAMP-response element; CREB, CRE-binding; TNF, tumor necrosis factor

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Received 5 July 2010 **Revised** 6 October 2010
Accepted 18 October 2010

Introduction

Chromogranin A (CHGA) is a 48-kDa acidic glycoprotein stored in and released from the chromaffin granules of endocrine cells, neurons, and neuroendocrine cells along with their respective hormones, neurotransmitters, and neuropeptides [1]. It has been clearly shown that CHGA is necessary for granule biogenesis, for the intracellular compartmentalization, and for secretion of hormones and neuropeptides [2,3]. The adrenergic vesicles of the adrenal medulla contain high concentrations of catecholamines (0.6 mol/l), Ca^{2+} (40 mmol/l), as well as remarkable amounts of CHGA (4 mmol/l) [4]. CHGA seems to bind both catecholamines and Ca^{2+}. The adrenal medulla is supposed to be the main source of circulating CHGA. On the contrary, adrenergic nerve endings and neuroendocrine cells secrete CHGA in peripheral tissues. The factors governing the pattern of expression of the CHGA gene have been only partially explored. The proximal promoter regions of the gene contain a cyclic adenosine monophosphate (cAMP)-response element (CRE) located upstream of a region rich in thymidine and adenine, referred to as TATA box. These upstream promoter elements are important in determining the extremely variegated cell-to-cell and species-to-species differences in the regulation of the expression of CHGA. These CRE regions, activated by the CRE-binding (CREB) transcription factor, are responsible for the upregulation of CHGA. Using the PC12 cell model system, it has been shown that both calcium and cAMP-stimulated CREB-dependent transcription via a Rap1 (Ras-related small G-protein)-B-Raf (neuronal Raf isoform)-ERK pathway. Agents that affect CHGA expression through cAMP include cholinergic agonists, neurotropoin nerve growth factor, and steroid hormones [1,5].

Numerous pairs of basic amino acids in the structure of CHGA indicate potential sites for cleavage by the prohormone convertases PC1/3 and PC2 that occur as costored components of the neurosecretory granules. These enzymes are released along with CHGA upon stimulation by the same secretagogues that govern CHGA release [6]. Although an endocrine role for CHGA remains to be established, its proteolytic fragments (Fig. 1) have been shown to exert a broad spectrum of regulatory activities on the cardiovascular, endocrine, and immune systems (Fig. 2) [7]. Among them, pancreastatin (human CHGA 250–301) inhibits insulin release from the pancreatic-islet β cells, promotes hepatic glycogenolysis, decreases glycogen synthesis and glucose uptake by skeletal muscles, and regulates lipid metabolism; prochromacin (bovine CHGA 79–431) and chromacin (human CHGA 176–197) exert direct antibacterial and antifungal effects; catestatin (human CHGA 352–372) inhibits catecholamine release from the cells of the adrenal medulla; vasostatin 1 (human CHGA 1–76) and vasostatin 2 (human CHGA 1–113) play a role in the regulation of the function of the heart and the vascular system, in physiologic conditions and pathologic disorders, as in the case of hypertension, heart failure, and vascular inflammatory diseases [7].

Among all known proteolytic peptides, only catestatin has been reported to act via a classical receptor type: the

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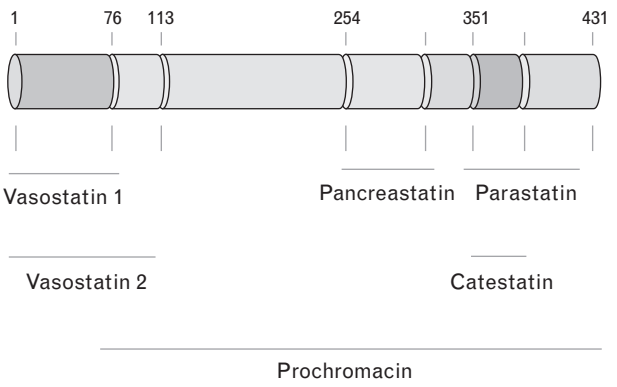
Fig. 1



Representation of the main sites of cleavage of chromogranin A by the prohormone convertases PC1/3 and PC2, resulting in several biologically active peptides.

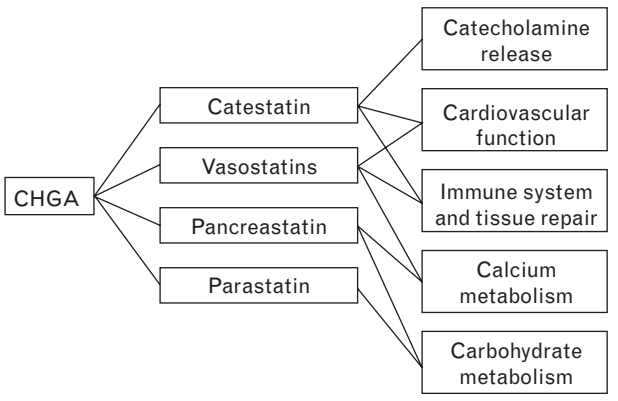
nicotinic acetylcholine receptor expressed on peripheral neuronal and neuroendocrine cells [8]. The molecules or mechanisms mediating the activity of the remaining peptides and CHGA itself are yet to be described.

In this article we will review the multifaceted activity of CHGA and its proteolytic fragments on the heart and the vascular tree, in health and pathologic conditions.

The control of blood pressure

The first association between CHGA and human arterial hypertension was done in 1985, when O'Connor and colleagues [9] observed that untreated patients with essential and several varieties of secondary hypertension had higher levels of circulating CHGA as compared with healthy individuals. They showed that the short-term suppression of the sympathetic outflow with oral drugs reduced plasma CHGA, suggesting that its level in the blood was under neural control. Subsequently, studies in monozygotic and dizygotic twins showed that plasma CHGA levels had a significant genetic variance and a high heritability. Interestingly, in established essential hypertension patients, CHGA responses to adrenal medullary and sympathetic neuronal activation were exaggerated, whereas responses to sympathoadrenal suppression were diminished, supporting the hypothesis of the adrenergic origin of the augmented circulating CHGA in this disorder [10]. It has been recently reported that a naturally occurring human genetic variation in the 3′-untranslated region of the CHGA gene is associated with increased arterial blood pressure and that this variant affects catecholamine storage in adrenergic vesicles [11]. CHGA is essential for the biogenesis of dense-core secretory vesicles in the adrenal chromaffin cells and adrenergic neurons by virtue of its pH, calcium, and catecholamine-dependent aggregation properties. It binds catecholamines driving their release. CHGA expression in adrenergic cells is under the control of the same factors regulating catecholamine synthesis and release. In particular, acetylcholine is the main secretagogue. It binds the nicotinic cholinergic receptors, triggering cAMP production and calcium influx, ultimately resulting in the exocytotic release of the chromaffin granules and in the transcription of the CHGA gene [1].

Fig. 2



The pleomorphic effects of chromogranin A proteolytic fragments in physiology.

However, the link between CHGA and catecholamine release, affecting blood pressure control, is more complicated. In fact, it has been shown that CHGA null mice develop elevated blood pressure as compared with wild type mice, a finding that seems to be in contrast with the aforementioned observations [12]. The explanation for this apparent contradiction comes from the protean activities of CHGA fragments [13]. In 1988, after fragmentation of bovine CHGA and conditioning of acetylcholine stimulated adrenal medullary chromaffin cells, it was observed for the first time that CHGA-derived peptides could inhibit catecholamine release [14]. It was later described that the bovine CHGA 344–364 peptide was able to block in-vitro catecholamine secretion from rat pheochromocytoma PC12 cells activated by acetylcholine [8]. This molecule (corresponding to human CHGA 352–372) was given the name catestatin, reflecting its property, that was further confirmed in other cell types and animal species [15]. Catestatin acts by binding the nicotinic acetylcholine receptor and its effect is noncompetitive as nicotine is not able to overcome it at any dose [16]. Interestingly, the effect of catestatin is specific as the stimulation of catecholamine release by several triggers other than acetylcholine is not affected [8]. The same observations were confirmed *ex vivo* and *in vivo* by stimulation of the splanchnic nerve in the rat [17]. These evidences suggest that catestatin acts on the nicotinic cholinergic receptors as an autocrine-negative feedback to control the indiscriminate release of catecholamines in the bloodstream. Consistently, the

increased levels of blood pressure in CHGA null mice could be reverted by the exogenous administration of catestatin [12]. Furthermore, hypertensive patients and patients with normal blood pressure but with genetic risk of hypertension show decreased levels of catestatin as compared with controls [18]. Also hypertensive patients with end-stage renal disease had decreased levels of catestatin. Whether this is an effect of the organ damage or the cause of the hypertensive state has not been elucidated yet [19].

Heritability and genome-wide linkage studies identified novel genomic regions controlling CHGA secretion and catestatin processing [19,20].

Interestingly, it has been shown that the intravenous injection of catestatin in rats after electrical stimulation of the sympathetic outflow diminished blood pressure, also in the presence of α and β adrenergic blockade. Therefore, the regulatory role of catestatin on blood pressure implies the presence of additional mechanisms, other than catecholamine inhibition. Accordingly, catestatin displays potent vasodilatory effects by increasing the secretion of histamine, possibly by stimulation of mast cells [21]. Arterial infusion of human catestatin in the forearm of 14 healthy volunteers caused a modest but significant increased in the blood flow [22].

Vasostatins, namely vasostatin 1 and vasostatin 2, are the N-terminal fragments of CHGA and are closely related to the control of blood pressure, through the regulation of the vascular tone. The vasodilator effect of vasostatins was first observed in 1992, using precontracted isolated segments of the human internal thoracic artery and of the saphenous vein. This effect was shown to be independent of the presence of the endothelium and, in the vein, dependent on extracellular calcium [23]. Subsequent reports supported this observation and showed that these molecules act through the inhibition of the potent vasoconstriction elicited by endothelin 1 [24,25]. Interestingly, the 1–40 fraction of vasostatin 1 maintained the inhibitory effect on vasoconstriction induced by noradrenaline but not by endothelin 1, suggesting a double mechanism related to the N-terminal and C-terminal regions of vasostatin 1. This observation was confirmed on the bovine coronary circulation [26].

Taken together, these studies reveal a role for CHGA on the regulation of arterial blood pressure, through its direct control on catecholamine secretion, and through the effect of its fragments catestatin and vasostatin 1 and 2, acting respectively on the release of soluble factors and on the control of the vascular tone.

Chromogranin A in the diagnosis of secondary systemic arterial hypertension

CHGA has been advocated as a marker to identify pheochromocytoma in the differential diagnosis of secondary arterial hypertension. In particular, it has been suggested as a powerful alternative to catecholamine measurement, as CHGA blood levels are not influenced by drugs commonly used in the treatment of pheochromocytoma, including all antihypertensive drugs. Its sensitivity is relatively high (86%), but its value as a diagnostic tool in the clinical setting is yet a matter of debate [27]. A main limitation to its use is the fact that CHGA is cleared by the kidneys and even mild degrees of renal impairment can lead to significant increases in the serum concentration of CHGA. Therefore, the specificity of CHGA measurement in patients with pheochromocytoma is low. In a study aimed at assessing the value of common biochemical tests in the diagnosis of pheochromocytoma, the authors found that the overall specificity of serum CHGA was 74%, but among patients with creatinine clearance less than 80 ml/min, the specificity decreased to 50% and the positive predictive value was 38%. However, when combined with elevated plasma catecholamines in hypertensive patients with creatinine clearance higher than 80 ml/min, the diagnostic specificity and positive predictive values of CHGA measurement improved to 98 and 97%, respectively [28].

Another matter of debate is the value of CHGA in distinguishing benign from malignant forms of pheochromocytoma. In a study on 13 patients with benign pheochromocytoma, evaluated before and after surgical excision, and on 14 patients with malignant pheochromocytoma, studied before and after conventional chemotherapy, the authors showed that CHGA levels rose progressively from control individuals (48.0 ± 3.0 ng/ml) to benign pheochromocytoma (188 ± 40.5 ng/ml) to malignant pheochromocytoma (2932 ± 960 ng/ml). CHGA was significantly different between benign and malignant pheochromocytoma. After excision of benign pheochromocytoma, CHGA, norepinephrine, and epinephrine levels fell to values near normal, whereas after chemotherapy of malignant pheochromocytoma, only CHGA and norepinephrine, but not epinephrine, were shown to decrease. Plasma CHGA varied longitudinally with tumor response and relapse [29]. Contrasting observations come from a study evaluating the value of plasma markers for the clinical behavior of pheochromocytoma. The authors found that larger pheochromocytomas, particularly those showing necrosis, capsular and vascular invasion, secreted higher levels of catecholamines but not of CHGA. In addition, they observed that patients with higher levels of dopamine, norepinephrine, and aromatic L-amino acid decarboxylase as well as lower ratios of epinephrine over norepinephrine had significantly shorter metastases-free intervals. CHGA was shown to add no significant information in terms of predicting malignant behavior in pheochromocytoma [30].

The control of the heart response

CHGA exerts significant regulatory activities on the cardiac function. Not just it can reach myocardial cells

from the bloodstream and adrenergic terminals, it has also been shown that the myocardium is able to produce and release CHGA, presumably contributing to the local modulation of its own response [31]. In the rat heart, CHGA is stored in nonadrenergic myoendocrine atrial cells containing atrial natriuretic peptide and in Purkinje fibers of the atrium and ventricle containing the calcium channel α1E subunit [32,33].

In 2002 Imbrogno *et al.* [34], studying the effects of exogenous vasostatin 1 and 2 on the isolated working frog heart, observed a significant calcium-dependent negative inotropism, involving neither the endocardial endothelium nor the adrenergic and muscarinic receptors. This was the first description of the cardiosuppressive role for the vasostatins. Subsequent reports extended this observation to the heart of the eel, showing the obligatory role of nitric oxide signaling in mediating the vasostatin response and, therefore, the need of an intact endocardial endothelium. In 2006, the same effects were observed on the heart of mammalians, by using the isolated Langendorff-perfused rat heart, that allows the examination of inotropic and chronotropic effects and of vascular effects without the neuronal and hormonal complications of an intact animal model. In the Langendorff preparation, the aorta is cannulated and the heart is perfused in a retrograde fashion. Cardiac performance was evaluated by analyzing left ventricular pressure and the rate pressure product, respectively, as indexes of contractile activity and cardiac work. Vasostatins 1 and 2 under basal conditions elicited a dose-dependent negative inotropism, affecting coronary pressure only at higher concentrations. Both molecules counteracted the cardiostimulatory effect of isoproterenol, whereas chronotropism was reduced only by vasostatin 2 [35]. More recently, it was shown in the eel and frog hearts that the vasostatin-mediated negative inotropic effect was abolished by treatment with inhibitors of cytoskeleton reorganization, suggesting that changes in cytoskeletal dynamics are crucial for the control of the heart response [36].

In 2002, serum CHGA levels were measured in 160 patients with chronic heart failure. CHGA was significantly increased as compared with controls and the levels correlated with the New York Heart Failure (New York Heart Association) severity class. Class IV patients showed the highest serum levels. In addition, CHGA was revealed to be an independent predictive marker for mortality [37]. Accordingly, in a study on 217 patients with complicated myocardial infarction, assessing the association between plasma CHGA levels and time to hospitalization for heart failure or death, it was found that CHGA was a strong and independent prognostic indicator of the outcome [38]. A larger study conducted on 1268 patients with acute coronary syndromes, after a median follow-up of 92 months, revealed that CHGA concentration was strongly associated with long-term mortality, hospitalization for heart failure, and recurrent myocardial infarction [39]. Interestingly, in patients with chronic heart failure, CHGA did not correlate with hormones that are typically activated, including catecholamines, vasopressin, endothelins, and components of the renin–angiotensin system [40]. Instead, they correlated with the levels of tumor necrosis factor (TNF)α and TNFα receptors [41], and with the levels of brain natriuretic peptide (BNP) [31]. It has been shown that CHGA colocolizes with BNP in biopsies from patients with dilated cardiomyopathy and hypertrophic myopathy [31].

In conclusion, CHGA circulating levels in chronic heart failure seem to reflect myocardial inflammation and distension more than neuroendocrine autonomic activation. Presumably, CHGA has a protective effect on the myocardium preventing excessive work in stressful conditions.

Endothelial integrity and vascular inflammation

The observations in patients with heart failure pointed out a connection between TNFα mediated-inflammation and CHGA secretion. Currently, an increasing body of evidence supports the concept that CHGA plays a protective effect on the inflamed endothelium. In-vivo experiments in mice and in-vitro experiments in a monolayer of human umbilical vein endothelial cells showed that CHGA inhibits endothelial leakage induced by TNFα, preventing cytoskeleton rearrangements [42,43]. Subsequent studies found that vasostatin 1 inhibited TNFα induced-gap formation in two model systems of vascular leakage in arterial endothelial cells of bovine pulmonary and coronary origin, through a p38MAPK (mitogen-activated protein kinase) signaling cascade and presumably a Gαi-coupled mechanism [44]. In addition, CHGA favored endothelial cell homeostasis inhibiting thrombin and vascular endothelial growth factor-mediated activation, including the effects on cell migration, proliferation, morphogenesis, and invasion of collagen gels [45].

Recently, we studied patients with several rheumatologic disorders that are associated with chronic vascular inflammation, and we described high levels of plasma CHGA as compared with age-matched and sex-matched healthy controls. In patients with rheumatoid arthritis, CHGA plasma levels correlated with the levels of TNFα receptors and treatment with anti-TNFα monoclonal antibodies abrogated this correlation, suggesting a complex connection between these two molecule systems [46]. Interestingly, patients with extra-articular manifestations showed extremely higher levels as compared with patients with joint-limited disease. CHGA levels were even higher than those observed in patients with neuroendocrine malignancies. Extra-articular manifestations in rheumatoid arthritis are known to be associated with an increased risk of death by cardiovascular events, presumably due to a sustained systemic vascular inflammation.

We demonstrated that recombinant vasostatin 1 and sera from patients with high levels of circulating CHGA were able to inhibit TNFα-mediated endothelial inflammation *in vitro* and that this effect was reverted by the use of antisera to vasostatin 1 [47]. This was the first demonstration of a protective effect of CHGA, through its N-terminal fragment vasostatin 1, on the vasculature in inflammatory disorders. Furthermore, we described significantly higher levels of CHGA in patients with temporal arteritis, a prototypic form of chronic autoimmune vasculitis. The highest levels were observed in patients refractory to conventional treatment, therefore presenting with persistent vascular inflammation [48]. In both rheumatoid arthritis and temporal arteritis the levels of circulating CHGA correlated neither with acute phase reactants, including C-reactive protein and erythrosedimentation rate, nor with validated clinical scores of disease activity. In addition, histology of biopsy samples of temporal arteries from patients with high plasma CHGA levels showed the absence of this molecule at the site of inflammation. Taken together, these observations support the concept that CHGA circulating levels do not depend on the extent of tissue inflammation and overall disease activity and that peripheral tissues are not the source of increased CHGA. The levels of circulating CHGA seem to reflect diffuse endothelial inflammation, even when disease seems under control according to common clinical scores. Large controlled clinical trials are necessary to validate the power of the measurement of CHGA in rheumatoid arthritis and temporal arteritis to predict vascular complications irrespective of the overall clinical activity.

In conclusions, our studies and previous observations support the hypothesis that CHGA is systemically secreted at high levels in chronic inflammatory conditions and that it exerts a protective effect over the inflamed endothelium, preventing excessive activation leading to vascular damage.

Conclusion

CHGA is a ubiquitous molecule mainly produced by adrenergic and neuroendocrine cells. After secretion, it gives rise to several proteolytic fragments that are concurrently active on the control of blood pressure and of the heart function. Not only previous studies on CHGA gave an interesting insight into the molecular bases of this disorder, but also proved the value of this molecule in the clinical setting, to detect and predict the risk of hypertension, the extent of heart dysfunction and the presence of chronic vascular inflammation in several inflammatory disorders. To date, no studies in humans have investigated CHGA and its fragment catestatin as potential treatment in systemic arterial hypertension. In-vivo studies conducted in CHGA knockout mice revealed that the intravenous injection of catestatin rescued animals from elevated blood pressure. In addition, epidemiologic studies on patients with primary and secondary hypertension showed that CHGA levels, and consequently catecholamine storage in the adrenal medulla, were decreased as compared with controls. These data seem promising in considering catestatin as a possible treatment in systemic arterial hypertension, at least in patients with increased levels of circulating catecholamines.

Acknowledgement

There are no conflicts of interest.

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