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Commentary

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Chromogranin A: a surprising link between granule biogenesis and hypertension

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Chromogranin A (CHGA) and its derived peptides, which are stored and released from dense-core secretory granules of neuroendocrine cells, have been implicated as playing multiple roles in the endocrine, cardiovascular, and nervous systems. In this issue of the *JCI*, Mahapatra et al. present in vivo evidence for 2 important functions of CHGA: the regulation of catecholamine-containing dense-core chromaffin granule biogenesis in the adrenal gland and the control of blood pressure (see the related article beginning on page 1942). Obliteration of CHGA expression in a KO mouse model led to decreased size and number of chromaffin granules as well as hypertension in these animals. Transgenic expression of human *Chga* and exogenous injection of human catestatin, a CHGA-derived nicotinic cholinergic antagonist, restored normal blood pressure in these mice. These results suggest a coupled relationship between CHGA-mediated chromaffin granule biogenesis, necessary for catecholamine storage, and catestatin-induced inhibition of cholinergic-stimulated catecholamine release, which regulates autonomic control of blood pressure.

Chromogranin A (CHGA) was identified as a major soluble protein in adrenal medullary chromaffin granules almost 4 decades ago (1). Since then it has been found to be a ubiquitous protein stored in dense-core secretory granules of the endocrine, exocrine, and nervous systems, along with their respective hormones, enzymes, and neuropeptides and neurotransmitters. CHGA and its derived peptides have been intensely studied with respect to their physiological roles and pathological expression in tumors. CHGA and its processed products have been shown to be involved in the biogenesis of dense-core secretory granules (2), to have a role in immunity against microbes (3), and to function as potential markers for several types of tumors (4). They have also been implicated in neurodegenerative disorders and cardiovascular diseases such as hypertension (4–6). However, these proposed functions of CHGA have not previously been verified in vivo. In this issue of the *JCI*, Mahapatra et al. (7) used a mouse model with genetic ablation of *Chga* gene expression to provide the first in vivo evidence to our knowledge for the distinct yet intertwined actions of full-length CHGA and its processed peptide catestatin in the autonomic control of the circulatory system through regulation of chromaffin granule biogenesis and catecholamine release from the adrenal gland.

Role of CHGA in regulating secretory granule biogenesis

Formation of dense-core secretory granules is a critical step in the storage and sequestration of bioactive molecules from (neuro)endocrine cells such as the chromaffin cells in the adrenal medulla. In chromaffin cells, catecholamine and ATP are bound to CHGA and are together packaged with proenkephalin and neuropeptide Y in dense-core chromaffin granules. Previous studies using cell lines have demonstrated a role for CHGA and other members of the granin family in the physical formation of the granules. When CHGA and chromogranin B (CHGB) were expressed in nonendocrine cells, dense-core secretory granule–like vesicles formed within the cells (2, 8, 9), which suggests that proteins with these aggregative properties are granulogenic (i.e., they can physically initiate granule formation). However, the ability to regulate the number of granules formed in neuroendocrine cells is unique to CHGA. In PC12 cells, a neuroendocrine cell line, the depletion of CHGA, but not CHGB, severely impaired granule formation (2). Concomitantly, other granule proteins such as CHGB, carboxypeptidase E (CPE), and synaptotagmin were degraded in the absence of CHGA in these cells. However, transfection of CHGA into these cells rescued the degradation of these proteins. It was therefore proposed that CHGA may regulate the number of granules formed by controlling the stability and availability of granule proteins at the posttranslational level (10). Indeed, the data from *Chga* KO mice presented by Mahapatra et al. (7) provide evidence in support of such a role for CHGA in regulating dense-core secretory granule biogenesis in vivo. These mice showed decreased numbers of granules formed in adrenal chromaffin cells lacking CHGA. In addition, morphological analysis of the granules formed revealed that they were significantly altered, showing a decrease in their size and electron density. Moreover, the expression levels of other granule proteins such as CHGB and dopamine β-hydroxylase were decreased. These results are strikingly similar to the ex vivo evidence obtained from using CHGA-deficient PC12 cells (2) and verify the importance of CHGA in regulating granule biogenesis in vivo. Furthermore, we have recently obtained similar results in a mouse model expressing antisense RNA against CHGA to partially deplete CHGA expression. In this transgenic mouse model, reduction of CHGA levels resulted in a significant decrease in the number of dense-core granules as well as a reduction in CHGB and dopamine β-hydroxylase levels in chromaffin cells (our unpublished observations). While there was some controversy and debate over the role of CHGA in granule biogenesis based on studies by several groups using cell lines (11, 12), the evidence from the *Chga* KO mice reported in this issue by Mahapatra et al. (7) and from our transgenic mouse model (our unpublished

Nonstandard abbreviations used: CHGA, chromogranin A; CPE, carboxypeptidase E.

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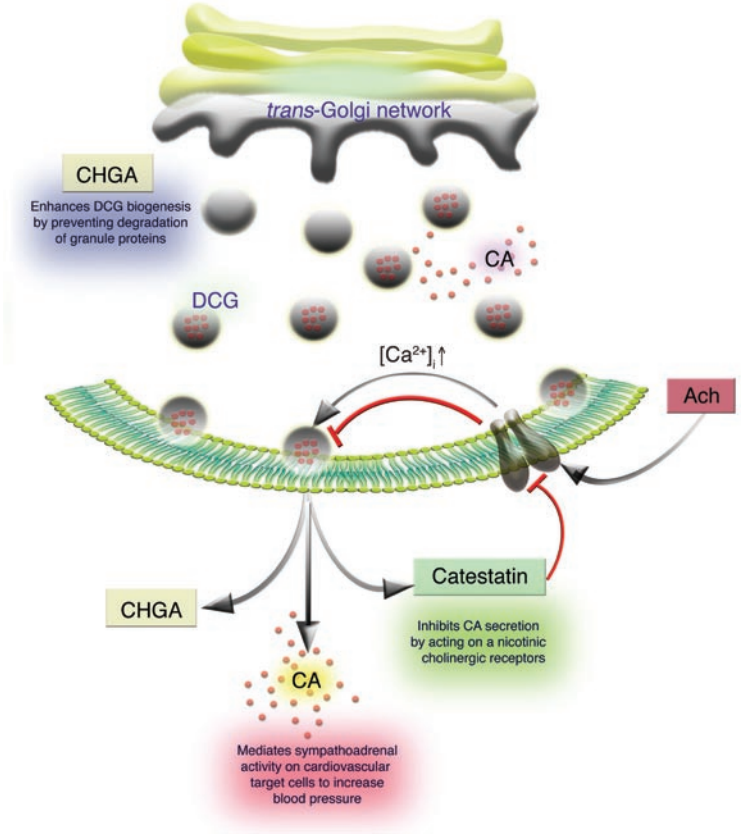

Figure 1
Relationship of CHGA-mediated dense-core secretory granule (DCG) biogenesis, catecholamine (CA) secretion, and its subsequent inhibition by the CHGA-derived peptide catestatin in the maintenance of blood pressure by the adrenal gland. CHGA, as a full-length molecule, initiates dense-core secretory granule biogenesis at the *trans*-Golgi network of adrenal chromaffin cells. Current data suggests that CHGA enhances granule biogenesis by preventing posttranslational degradation of other granule proteins in the Golgi complex. In the cytoplasm, catecholamine is synthesized and transported into the dense-core secretory granules via vesicular monoamine transporters. Upon stimulation by acetylcholine (Ach), catecholamine is coreleased with CHGA and catestatin from the granules. Secreted catecholamine triggers cardiovascular target cells to augment blood flow. This sympathoadrenal activity is then antagonized by the action of catestatin on cholinergic receptors to inhibit catecholamine secretion. $[Ca^{2+}]_i$, intracellular calcium concentration.

observations) unequivocally points to an essential physiological role for CHGA in dense-core secretory granule biogenesis in adrenal chromaffin cells.

Partial embryonic lethality was reported for the *Chga*$^{-/-}$ homozygotes, which indicated severe abnormality caused by CHGA depletion (7). Therefore, those *Chga*$^{-/-}$ homozygotes that lived must possess compensatory mechanisms for survival despite the deletion of *Chga* gene. In fact, the mRNAs encoding several chromaffin granule proteins including CHGB, secretogranin II, vesicular monoamine transporter-1 and -2, and dopamine β-hydroxylase were significantly increased in *Chga*$^{-/-}$ mice. As the authors discussed in their study, the elevated corticosterone levels in *Chga* KO mice, which are known to upregulate *Chga* gene expression in adrenal chromaffin cells, may be considered a compensatory mechanism to overcome the lack of CHGA. The increase of mRNAs for other granule proteins may be a side effect of the increased glucocorticoid levels in these animals. Nevertheless, the total levels of CHGB and dopamine β-hydroxylase gene products in the adrenals were significantly decreased in *Chga* KO mice. This result is reminiscent of the low levels of granule proteins CHGB, CPE, and synaptotagmin observed in PC12 cells lacking CHGA, which are due to degradation (2). The levels of granule proteins correlated with the amount of CHGA present in the PC12 cells and their degradation was recovered by re-expression of CHGA in these cells, which suggests that CHGA plays a role in regulating granule protein stability and hence affects the levels available for dense-core granule biogenesis. It remains to be determined whether these granule proteins are synthesized and degraded in the absence of CHGA in the *Chga* KO mouse.

CHGA and blood pressure regulation

Primary (genetic or essential) hypertension is complex, as multiple factors derived from cardiovascular, neuronal, renal, and adrenal sources contribute to it. For the last 20 years, a link between CHGA and hypertension has been postulated. Sympathoadrenal activity mediated by catecholamine acts on cardiovascular target cells to increase blood pressure by a so-called "flight or fight" response. After catecholamine is synthesized in the cytoplasm of chromaffin cells, it is transported into dense-core granules via vesicular monoamine transporters. Catecholamine then binds CHGA for storage in the core of the granules (13, 14). Upon stimulation by acetylcholine, catecholamine is cosecreted with CHGA and its derived peptide catestatin from granules in chromaffin cells (Figure 1). Secreted catecholamines in plasma augment blood pressure by activating cardiovascular target cells. Therefore, an increase in blood pressure caused by the action of catecholamine is tightly coupled to the formation of dense-core granules, which is regulated by CHGA. The release of catecholamine is then subsequently blocked by the cosecreted catestatin from chromaffin granules and acts as an antagonist at nicotinic cholinergic receptors, which thus alleviates high blood pressure (Figure 1) (4, 15). The development of hypertension in the *Chga* KO mice and their subsequent recovery through injection of catestatin or transgenic expression of human *Chga* clearly demonstrates a role for this peptide in inhibiting blood pressure increases (7). This is consistent with observations that hypertensive patients and subjects with normal blood pressure but with genetic risk of hypertension have decreased catestatin levels compared to those of normotensive controls (16). Interestingly, while catestatin levels were low in these patients, plasma CHGA levels and catecholamine excretion both increased (5, 17). One possible explanation for this result is that there is a processing defect of CHGA in these hypertensive patients.

Alternatively, these patients may undergo a pathological upregulation of cholinergic input, which could lead to enhanced exocytosis of chromaffin granules that have not fully matured and secretion of unprocessed CHGA, since processing of CHGA into catestatin takes place in the granules (18). Uninhibited stimulation of chromaffin granule exocytosis with lowered catestatin levels would account for the higher sympathoadrenal activity that leads to increased levels of circulating catecholamine and hypertension in these patients.

In conclusion, the study by Mahapatra et al. (7) describes a mechanism that links the requirement of CHGA for sustained chromaffin granule biogenesis and catecholamine storage to the extracellular inhibition of catecholamine secretion by the CHGA-derived peptide catestatin in the autonomic control of blood pressure in vivo (Figure 1). Their work has several clinical implications. One is the possibility that catestatin may be useful as a therapeutic agent in the treatment of hypertension in humans, although the extent to which the lack of catestatin contributes to human hypertension remains to be determined. Furthermore, screening for polymorphisms in the catestatin domain (19) of the *Chga* gene in humans could provide insight into the observed genetic predisposition to hypertension.

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