Brief Review

Chromogranin A
Storage and Release in Hypertension

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The chromogranins/secretogranins are a family of acidic, soluble proteins with widespread neuroendocrine distribution in secretory vesicles. Although the precise function of the chromogranins remains elusive, knowledge of their structure, distribution, and potential intracellular and extracellular roles, especially that of chromogranin A, has greatly expanded during recent years. Chromogranin A is co-released with catecholamines by exocytosis from vesicles in the adrenal medulla and sympathetic nerve endings. Thus, measurement of its circulating concentration by radioimmunoassay may be a useful probe of exocytotic sympathoadrenal activity in humans, under both physiological and pathological conditions. Here, we explore the storage, structure, and function of chromogranin A, and parameters that influence its circulating levels. We have also measured plasma chromogranin A concentrations in different groups of patients with hypertension, including those with pheochromocytoma. (Hypertension 1990;15:237-246)

In adrenal chromaffin cells and sympathetic nerve endings, catecholamines are stored in membrane bound organelles, the catecholamine storage vesicles, called chromaffin vesicles (granules) in the adrenal medulla.1,2 Catecholamine storage vesicles are apparent as electron dense core spherical or oblong structures when viewed by transmission electron microscopy, as shown by the human pheochromocytoma section in Figure 1.

Catecholamine Storage Vesicles

After homogenization of chromaffin tissue, chromaffin vesicles can be isolated from other subcellular organelles by virtue of their buoyant density on isopyknic sucrose gradients (Figures 2 and 3). When such vesicles are lysed hypotonically and the vesicle membranes removed by ultracentrifugation, the vesicle soluble cores, containing catecholamines, adenosine triphosphate, and soluble proteins and peptides,1 remain in the supernatant, and can be resolved by one- or two-dimensional gel electrophoresis (Figure 4).

In addition to dopamine β-hydroxylase, which is the enzyme catalyzing the biosynthetic conversion of dopamine to norepinephrine, the soluble proteins include a cluster of forms of sodium dodecyl sulfate (SDS) M, from ~50–120 kd and pI from ~4.5–5.2, which are the acidic chromogranins or secretogranins (Figure 4). The nomenclature of this family of proteins has been tentatively established.6 Other chromaffin vesicle soluble peptides not well visualized in Figure 4 include proenkephalin A and its derivatives (especially in epinephrine vesicles), prodynorphin and its derivatives (especially in norepinephrine vesicles), and neuropeptide Y.1

Chromaffin vesicle membrane proteins (not shown) include dopamine β-hydroxylase,7 cytochrome b-561, and glycoprotein III.1

In postganglionic sympathetic axons, there are both large (LDV) (75–90 nm diameter) and small (SDV) (45–55 nm diameter) dense core catecholamine storage vesicles.8 The large vesicles are qualitatively similar to chromaffin vesicles, except for their smaller dimensions (100–300 nm for chromaffin vesicles2 [see Figure 3] vs. 75–90 nm for LDV).8 The SDV lack dopamine β-hydroxylase and chromogranin A in their soluble cores.9

Chromogranins/Secretogranins

The chromogranins or secretogranins6,10 are a family of acidic, soluble proteins found in the core of chromaffin vesicles. Figure 4, a two-dimensional gel electrophoretic resolution of bovine chromaffin vesicle soluble cores, illustrates the relative abundances of chromogranin A (SDS M, ~70 kd, pI ~4.6–4.9) and chromogranin B (SDS M, ~120 kd, pI ~4.9–5.1), sometimes referred to as secretogranin I.6 Figure 4

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also illustrates products of proteolytic cleavage of each.

The chromogranins/secretogranins have a more widespread distribution than chromaffin vesicles; they are also found in postganglionic sympathetic neuronal catecholamine storage vesicles, and indeed, seem to be ubiquitous in virtually all hormone storage vesicles throughout the neuroendocrine system, where their function remains a matter of conjecture.

By convention, the parent SDS $M_r \sim 70$ kd form is referred to as chromogranin A (previously referred to on occasion as secretory protein I or parathyroid secretory protein), the parent SDS $M_r \sim 120$ kd form as chromogranin B (previously referred to as secretogranin I), and the parent SDS $M_r \sim 85$ kd form as secretogranin II (previously referred to as chromogranin C). Secretogranin II is quantitatively a minor component in chromaffin vesicles (not visualized, for example, in Figure 4) but is a major component of anterior pituitary hormone storage vesicles.

**Chromogranin A Structure**

The primary structures of bovine, rat, porcine, and human chromogranin A, as well as that of human and rat chromogranin B and secretogranin II (chromogranin C), have been determined. Comparison of the amino acid sequence of human chromogranin A with that of bovine, porcine, and rat chromogranin A shows sequence conservation at the N- and C-terminals, with a good deal of mid-molecular sequence interspecies divergence. In
humans, the chromogranin A gene, shown to be a single gene on Southern blot analysis, has been localized to chromosome 14 by analysis of flow-sorted chromosomes.

Figure 5 is a domain map based on the complementary DNA (cDNA)-deduced primary structure of human chromogranin A. The chromogranin A molecule contains the following putative structural/functional domains: a hydrophobic N-terminal signal peptide, which is cleaved from the mature protein; 10 pairs of basic amino acid residues, potential cleavage, or processing sites; two cysteine residues toward the N-terminus, which may form an intramolecular disulfide loop; an interior amino acid sequence identical to the insulin release-suppressing peptide pancreastatin; areas of homology to known calcium binding proteins such as oncomodulin, brain S-100 protein, and intestinal calcium binding protein; and an arg-gly-asp (RGD) that may be involved in cell membrane attachment.

In solution, chromogranin A assumes a structureless random coil conformation, as suggested by its low percent a helix coupled with its low sedimentation coefficient, large Stokes radius, and highly acidic amino acid composition and pI.

Table 1 displays some comparative selected properties of human chromogranins A and B and secretogranin II (chromogranin C), whose primary structures are known.

In human pheochromocytoma chromaffin vesicle cores, size-separated by one-dimensional sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and subjected to immunoblotting (Figure 6), it can be seen that chromogranin B is relatively prominent in these vesicles in contrast to normal bovine chromaffin vesicles.

Chromogranin A Function

Although the primary structure of chromogranin A is now known, its physiological role remains in debate.

No evidence for a direct action of chromogranin A in modulating blood pressure has been found; neither intravenous nor intracerebroventricular injection of purified bovine chromogranin A into rats induced a change in blood pressure or heart rate.

On the other hand, an intracellular functional role for chromogranin A has been suggested by its ability to 1) bind calcium; 2) bind catecholamines; 3) complex adenosine triphosphate; 4) function as an ion exchanger; 5) inhibit a prohormone processing serine protease, and thus serve as an intravesicular modulator of hormone processing; and 6) diminish effective osmotic pressure in the intact chromaffin vesicle, thus stabilizing the vesicle against rupture.

By using synthetic peptides corresponding to different domains of the chromogranin A molecule and their corresponding polyclonal antibodies, we, as well as others, have demonstrated that chromogranin A is processed within the chromaffin vesicle by cleavage proceeding from both N- and C-terminals. As a result of this cleavage, chromogranin A may serve as a prohormone. Chromogranin A has 8–10 sites of paired basic residues, possible points of cleavage and liberation of putative biologically active peptides. Indeed, an interior fragment of chromogranin A is identical in sequence to pancreastatin, a peptide capable of suppressing pancreatic insulin release. This supports the potential functional role for chromogranin A as a prohormone capable of yielding smaller physiologically active peptides, including pancreastatin. In bovine chromaffin cell cultures, chromogranin A–derived peptides may inhibit nicotinic cholinergic agonist–mediated catecholamine release, providing potential homeostatic control on exocytosis.

Chromogranin A: Parameters Influencing Its Circulating Concentration

In vitro and in experimental animals, chromogranin A is costored and coreleased with catecholamines.
from vesicles in the adrenal medulla and sympathetic nerves,\textsuperscript{48,49} providing physiological evidence for exocytosis as the mode of catecholamine secretion.

Chromogranin A can be measured by radioimmunoassay in humans\textsuperscript{50}; the circulating immunoreactivity is remarkably stable, demonstrating resistance to lyophilization (Figure 7, left panel), repeated freezing and thawing (Figure 7, right panel), and prolonged heating at 37°C (up to 4 days). It does not circulate in close association with other plasma proteins (Figure 8).

Plasma chromogranin A responds to physiological manipulations of exocytotic sympathoadrenal activity. Selective, intense stimulation of either the adrenal medulla (insulin hypoglycemia) or sympathetic nerve endings (vigorous dynamic exercise) induces measurable increments in plasma chromogranin A along with plasma catecholamines.\textsuperscript{51,52} However, the magnitude of the change in plasma chromogranin A concentration during sympathetic neuronal stimulation is considerably less than that attained during adrenomedullary stimulation.\textsuperscript{53} This is consistent with the finding that human sympathetic axons contain approximately 97-fold less chromogranin A (\(\mu g/g\)) than the adrenal medulla.\textsuperscript{53}

In contrast, less intense sympathoadrenal stimulation (caffeine ingestion, standing, smoking, and low intensity exercise) moderately elevates plasma catecholamines but is insufficient to perturb the relatively high prevailing concentrations of chromogranin A.\textsuperscript{53}

Pharmacological stimuli to nonexocytotic catecholamine release (tyramine, reserpine) do not alter...
plasma chromogranin A (personal observations), reinforcing the notion that its plasma concentration is coupled to exocytosis, rather than to catecholamine release in general.

By immunohistology and radioimmunoassay, chromogranin A exhibits a widespread neuroendocrine distribution but is not localized to nonpeptide producing endocrine tissues or to exocrine tissues. Within the neuroendocrine system, its most abundant cellular source is the adrenal medulla.

In normal humans, individual provocation of endocrine glands (i.e., adrenal medulla, anterior pituitary, pancreatic islet, gut endocrine, parathyroid chief, and thyroid parafollicular C-cells) with appropriately tissue-selective secretagogues results in measurable release of the usual resident hormone (e.g., glucose causing pancreatic β cell insulin and C-peptide release), but only adrenal medullary stimulation also elevates plasma chromogranin A consistent with the finding that the adrenal medulla is the quantitatively major normal tissue source of chromogranin A.

The predominant source or sources of basal or unstimulated plasma chromogranin A in humans are still a matter of investigation. Human chromogranin A exhibits a significant ultradian or pulsatile rhythm, peaking on average every 51 minutes. Somatostatin infusion in humans suppresses basal circulating levels of chromogranin A and diminishes the frequency and amplitude of pulsatile peaks of plasma chromogranin A; however, plasma catecholamines are unchanged, suggesting that somatostatin either inhibits chromogranin A release from nonsympathoadrenal sources or interferes with transport of chromogranin A to the circulation. In experimental animals, released adrenal medullary chromogranin A arrives in the circulation in part via a route involving the lymphatics.

In renal insufficiency, plasma chromogranin A (as immunoreactive fragments) is elevated in proportion to the degree of uremia. This suggests that the kidney is involved in chromogranin A removal from circulation. Consequently, assessment of renal function is required for proper interpretation of elevations in plasma chromogranin A. On the other hand, chromogranin A is elevated to a lesser degree in patients with even severe liver disease, precluding a quantitatively major role for the liver in chromogranin A disposition.

Adults with uncomplicated diabetes mellitus have normal circulating chromogranin A, arguing against an important role for systemic chromogranin A (a precursor of pancreastatin) in the altered insulin release seen in this disorder.
TABLE 1. Comparative Properties of Human Chromogranin A, Chromogranin B, and Secretogranin II (Chromogranin C)

<table>
<thead>
<tr>
<th>Property</th>
<th>Chromogranin A</th>
<th>Chromogranin B</th>
<th>Secretogranin II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight (daltons)</td>
<td><strong>68,000</strong></td>
<td><strong>120,000</strong></td>
<td><strong>85,000</strong></td>
</tr>
<tr>
<td>By SDS gel electrophoresis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>By cDNA sequence</td>
<td>48,918</td>
<td>76,295</td>
<td>70,868</td>
</tr>
<tr>
<td>Isoelectric point</td>
<td>4.57-4.68</td>
<td>5.3-5.7</td>
<td>4.8</td>
</tr>
<tr>
<td>N-terminal hydrophobic signal peptide, residue number</td>
<td><strong>−18 to −1</strong></td>
<td><strong>−20 to −1</strong></td>
<td><strong>−30 to −1</strong></td>
</tr>
<tr>
<td>Basic amino clusters, sets of two or more</td>
<td>10</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>RGD (−arg−gly−asp−), residue number</td>
<td>43-45</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Oligo-glu clusters, number of sets (three or more residues)</td>
<td>6</td>
<td>4</td>
<td>None</td>
</tr>
<tr>
<td>Cysteines, residue number</td>
<td>17,38</td>
<td>16,37</td>
<td>None</td>
</tr>
<tr>
<td>Pancreastatin domain, residue number</td>
<td>250-301</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Asp+glu, % of total residues</td>
<td>26.4</td>
<td>23.6</td>
<td>19.3</td>
</tr>
</tbody>
</table>

SDS, sodium dodecyl sulfate; cDNA, complementary DNA.

Circulating Chromogranin A in Endocrine Neoplasia

By immunohistology and immunoblotting, chromogranin A is found in most peptide-producing endocrine neoplasms with dense core secretory vesicles; among the chromograninsSECRETOGRANINS, chromogranin A may have the most widespread occurrence in such neoplasms. Plasma chromogranin A is elevated in a variety of endocrine neoplasms including pheochromocytoma, aortic body tumor, carcinoid tumor, pancreatic islet cell tumor, oat cell lung carcinoma, medullary thyroid carcinoma, and parathyroid adenoma and hyperplasia. In these neuroendocrine neoplasms, the diagnostic sensitivity and specificity of plasma chromogranin A have been estimated at 81% and 100%, respectively.

Recent reports also suggest plasma chromogranin A elevation by neuroblastoma and by a variety of pituitary tumors. In each of these neoplasia, peptide hormones are stored and released from secretory vesicles. By contrast, choriocarcinoma, which releases chorionic gonadotropin from a nonvesicular pool, does not elevate plasma chromogranin A, reinforcing the linkage between peptide hormone storage vesicles and the chromogranin/secretogranin family.

Chromogranin A in Hypertension

Measurements of plasma norepinephrine and epinephrine have been used to assess sympathoneuronal and adrenomedullary activity, respectively, in humans. In essential hypertension, increased basal sympathetic outflow, reflected by elevated plasma norepinephrine levels, and an exaggerated adrenomedullary catecholamine release in response to stress (hypoglycemia) have been reported. However, plasma catecholamines may not always reflect changes in sympathetic outflow.

Table 2 displays plasma chromogranin A concentrations in normal control subjects as well as in different groups of patients with hypertension. Plasma chromogranin A is elevated (~40%) in patients with untreated essential hypertension and...
FIGURE 7. In vitro stability of human plasma chromogranin A immunoreactivity. Left panel: Immunoreactivity before and after lyophilization plus water reconstitution (n=7 samples). Right panel: Immunoreactivity during repeated freeze/thaw cycles (n=6 samples).

FIGURE 8. Line graph showing lack of association of chromogranin A with other plasma proteins. Iodine-125-labeled purified human chromogranin A was gel filtered either before or after preincubation (37°C, 30 minutes) with 0.5 ml normal human plasma. Column was standardized for void volume (V₀) with blue dextran, total internal volume (Vₐ) with [mI]Na and KCl, and elution position of iodine-125-labeled chromogranin A.

TABLE 2. Plasma Chromogranin A in Normotensive Control Subjects and in Subjects With Renal Parenchymal Disease and With Different Forms of Hypertension

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age (yr)</th>
<th>SBP (mm Hg)</th>
<th>DBP (mm Hg)</th>
<th>pCgA (ng/ml)</th>
<th>SCr (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>20</td>
<td>47±4</td>
<td>126±4</td>
<td>75±2</td>
<td>54±3</td>
<td>0.9±0.1</td>
</tr>
<tr>
<td>Essential hypertension</td>
<td>25</td>
<td>52±2</td>
<td>142±2</td>
<td>94±2</td>
<td>74±7</td>
<td>1.1±0.1</td>
</tr>
<tr>
<td>Renovascular hypertension</td>
<td>9</td>
<td>64±1</td>
<td>159±4</td>
<td>90±10</td>
<td>75±10</td>
<td>1.5±0.1</td>
</tr>
<tr>
<td>Renal parenchymal disease</td>
<td>25</td>
<td>54±3</td>
<td>143±5</td>
<td>90±3</td>
<td>221±20</td>
<td>4.1±0.3</td>
</tr>
<tr>
<td>Pheochromocytoma</td>
<td>21</td>
<td>39±3</td>
<td>134±4</td>
<td>82±4</td>
<td>702±289</td>
<td>...</td>
</tr>
</tbody>
</table>

Values are expressed as mean±1 SEM. Hypertensive subjects were either untreated or were free of antihypertensive medications for a period of 2-14 days, except for pheochromocytoma subjects, who were all treated, usually with oral phenytoin either alone or in combination with other agents. Chromogranin A was assayed as previously described.9 SBP, systolic blood pressure; DBP, diastolic blood pressure; pCgA, plasma chromogranin A; SCr, serum creatinine.
correlates with diastolic pressure in grouped normal and essential hypertensive subjects (r=0.316, n=45, p=0.034), suggesting that an excess of exocytic sympathoadrenal tone may be involved in the initiation or maintenance of essential hypertension. The effects of renal insufficiency on chromogranin A are also shown in Table 2; chromogranin A rises to a similar extent in normotensive and hypertensive uremics.59 In essential hypertension, short term suppression of sympathetic tone with the central α₉ agonist guanabenz decreases blood pressure as well as basal plasma chromogranin A,35 demonstrating neural control of the plasma chromogranin A basal elevation in essential hypertension. On the other hand, therapy with either the angiotensin converting enzyme inhibitor enalapril or the β-blocker propranolol lowered blood pressure without changing plasma chromogranin A. Thus, during antihypertensive therapy, only sympatholytic agents, if any, are likely to affect plasma chromogranin A.

Schober et al17 have recently reported a significant increase in the content of chromogranins (including chromogranin A) as well as catecholamines in adrenal medullary chromaffin vesicles of spontaneously (genetic) hypertensive rats (SHRs) in comparison with the normotensive Wistar-Kyoto control strain. This increase in adrenomedullary content or storage of chromogranins in SHRs, a model of human genetic hypertension, provides a potential explanation for the observed plasma chromogranin A elevation in human essential hypertension.35 In patients with pheochromocytoma, plasma chromogranin A is markedly elevated (~10–20-fold) suggesting that catecholamine secretion from the tumor is at least in part exocytic. Furthermore, the elevation in plasma chromogranin A parallels tumor mass.62 Thus, plasma chromogranin A may be a useful diagnostic tool for pheochromocytoma and might be used to predict extent of disease as well as to assess response to treatment.

In conclusion, measurement of circulating chromogranin A has yielded new insights into exocytic sympathoadrenal activity in human hypertension. Studies in larger numbers of human hypertensive individuals will define the differential diagnostic value of chromogranin A in the evaluation of pheochromocytoma. The recent availability of the full-length primary structure of chromogranin A17-21 cDNAs that can be expressed and mutagenized, and synthetic peptides spanning the molecule’s putative structural domains45-46 (Figure 5) should yield clues in the near future to the functional importance of this major constituent of the catecholamine storage vesicle.

Acknowledgment

We appreciate the technical assistance of Annie Chen.

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KEY WORDS • chromogranins • catecholamines • adrenal gland • sympathetic nervous system • pheochromocytoma • essential hypertension
Chromogranin A. Storage and release in hypertension.
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Hypertension. 1990;15:237-246
doi: 10.1161/01.HYP.15.3.237

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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