The Extended Granin Family: Structure, Function, and Biomedical Implications

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The chromogranins (chromogranin A and chromogranin B), secretogranins (secretogranin II and secretogranin III), and additional related proteins (7B2, NESP55, proSAAS, and VGF) that together comprise the granin family subserve essential roles in the regulated secretory pathway that is responsible for controlled delivery of peptides, hormones, neurotransmitters, and growth factors. Here we review the structure and function of granins and granin-derived peptides and expansive new genetic evidence, including recent single-nucleotide polymorphism mapping, genomic sequence comparisons, and analysis of transgenic and knockout mice, which together support an important and evolutionarily conserved role for these proteins in large dense-core vesicle biogenesis and regulated secretion. Recent data further indicate that their processed peptides function prominently in metabolic and glucose homeostasis, emotional behavior, pain pathways, and blood pressure modulation, suggesting future utility of granins and granin-derived peptides as novel disease biomarkers. **(*Endocrine Reviews* 32: 755–797, 2011)**

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/er.2010-0027 Received November 10, 2010. Accepted July 14, 2011.
First Published Online August 23, 2011

Abbreviations: ALS, Amyotrophic lateral sclerosis; ARC, arcuate nucleus; BDNF, brain-derived neurotrophic factor; BP, blood pressure; CG, chromaffin granule; CgA, chromogranin A; CGRP, calcitonin gene-related peptide; CNS, central nervous system; COX, cyclooxygenase; CSF, cerebrospinal fluid; CST, catestatin; DCG, dense-core secretory granule; Gsα, α-subunit of the stimulatory G protein; icv, intracerebroventricular; IP3, inositol 1,4,5-triphosphate; IP3R, IP3 receptor; KO, knockout; LDCV, large dense-core vesicle; NERP, neuroendocrine regulatory peptide; NESP55, neuroendocrine secretory protein of Mr 55,000; NPY, neuropeptide Y; OA, osteoarthritis; PC, prohormone convertase; PG, prostaglandin; pI, isoelectric point; PKA, protein kinase A; PN-1, protease nexin 1; POMC, proopiomelanocortin; PST, pancreastatin; PVN, paraventricular nucleus of the hypothalamus; RA, rheumatoid arthritis; RER, rough endoplasmic reticulum; SgII, secretogranin II; SIRS, systemic inflammatory response syndrome; SN, secretoneurin; SNP, single-nucleotide polymorphism; SOD1, superoxide dismutase 1; TGN, trans-Golgi network; UTR, untranslated region; VEGF, vascular endothelial growth factor; VST, vasostatin; WE14, 14 amino acid peptide with N-terminal tryptophan (W) and C-terminal glutamatic acid (E).

I. Introduction

In this review, we discuss the advantages of considering granins as members of an extended but functionally conserved family, and detail the structure, biological activities, secretory pathway sorting, genetics, and diagnostic and prognostic utility of this unique group of secreted proteins and peptide precursors. Because we broadly review eight granin proteins and their peptides, concentrating on endocrine, neuroendocrine, and neuronal functions, several other areas of interest have not received in-depth coverage. Fortunately, a number of excellent recent reviews provide additional detail on the structures and activities of specific granins and granin-derived peptides; these have been cited throughout our review, and several are summarized in Table 1.

A. Regulated secretion

Hormones, growth factors, neuropeptides, processing enzymes, and catecholamines are just some of the proteins and neurotransmitters that are secreted from endocrine, neuroendocrine, and neuronal cells. Secretion can be constitutive, as it is for Ig release from B cells (1), but for many biologically active molecules, it is more likely to be highly regulated and coupled to the exposure of cells to specific secretagogues or to depolarization (2). Secretory proteins destined for the regulated secretory pathway enter the rough endoplasmic cisternae, are transported to the trans-Golgi network (TGN), and are then targeted into dense-core secretory granules (DCG), otherwise known as large dense-core vesicles (LDCV) or, in the adrenal medulla, chromaffin granules (CG). Targeting is mediated by receptors that control entry into the regulated pathway (sorting by entry) and/or by progressive condensation of regulated secretory proteins within the immature granule during maturation (sorting by retention) and the budding off of clathrin-coated vesicles that contain incorrectly sorted, constitutively secreted proteins (*e.g.*, furin) (3–5). This generates a pool of highly concentrated cargo, crystalline in the case of insulin (6, 7). Winkler coined the term vesicular cocktail to indicate the presence of a large variety of solutes inside the CG; cargo with concentrations as high as 1.8 mM for chromogranin A (8, 9) and 0.5–1 M for catecholamines (10) have been reported. An osmolarity of approximately 1500 mOsm has been estimated when ATP, Ca^{2+}, peptides, ascorbate, and other proteins including enzymes, hormones, and growth factors are additionally considered (11). The granule cargo is therefore protected within LDCV from forming a formidable osmotic load by its relative insolubility and condensation (12). This state of aggregation contributes to gradual dissipation of cargo, such as for prolactin (13), insulin (14, 15), and catecholamines (16), after their release from the cell. Other proteins targeted into the regulated secretory pathway granules include enzymes such as dopamine-β-hydroxylase (17), tissue plasminogen activator (18), and members of the prohormone convertase (PC) family that process precursor proteins, generating diverse, biologically active secreted peptides (19, 20). The signals that target proteins into the regulated secretory pathway and/or cause their retention in this pathway have been the subject of intense investigation (reviewed in Refs. 3, 4, 21, and 22) and extensive discussion in *Section III* of this review, yet generalizable sorting mechanisms for regulated protein export still remain elusive.

LDCV, which are generally 80–120 nm in diameter, are estimated to number 10,000–30,000 in a typical endocrine or chromaffin cell (23–26); a subset of these fuse to the cell's plasma membrane in response to a secretory stimulus (27, 28), sometimes releasing only a fraction of each vesicle's content through a transiently formed pore (29). Although the LDCV pool is large, and proteins can be stored for several days, mature LDCV in pancreatic β-cells containing the most recently synthesized insulin, for example, bud from the Golgi and translocate within minutes to positions closest to the plasma membrane, where they fuse and release their contents, often before the secretion of cargo from chronologically older LDCV (22).

B. Secretory granule biogenesis and content

Packaging of hormones, growth factors, enzymes, and catecholamines in LDCV requires a mechanism for secretory vesicle formation or biogenesis (discussed in *Section III*), that has been shown relatively recently to depend on several granin family members, chromogranin A (CgA), CgB, secretogranin II (SgII), and SgIII (30–33). Insight into secretory granule content was first obtained through

TABLE 1. Summary of recent and highly cited reviews on the extended granin family

Authors	Title	Ref.	Year	Content
ISI Web of Science top five cited reviews				
Winkler H, Fischer-Colbrie R	The Chromogranins A and B: the First 25 Years and Future Perspectives	9	1992	Commentary review of the first 25 yr after the first identification of CgA
Huttner WB, Gerdes HH, Rosa P	The Granin (Chromogranin/ Secretogranin) Family	369	1991	Review of chromogranins and secretogranins, addresses chemical utility of granin family, sorting, granulogenesis, and biomarker potential
Taupenot L, Harper KL, O'Connor DT	The Chromogranin-Secretogranin Family	43	2003	Review of the extended granin family (chromogranins, secretogranins, NESP55, 7B2, and HISL-19); primarily focuses on molecular and genetic aspects and their biomedical implications
Simon JP, Aunis D	Biochemistry of the Chromogranin-A Protein Family	370	1989	Biochemical properties of CgA and its derived peptides
Somogyi P, Hodgson AJ, DePotter RW, Fischer-Colbrie R, Schober M, Winkler H, Chubb IW	Chromogranin Immunoreactivity in the Central Nervous-System	371	1984	CNS immunoreactivity of CgA and its relation to other peptidergic and monoaminergic pathways
Other reviews covering the granin family of proteins				
Helle KB	The Granin Family of Uniquely Acidic Proteins of the Diffuse Neuroendocrine System: Comparative and Functional Aspects	44	2004	Review the extended granin family (CgA, CgB, SgII, SgIII, NESP55, 7B2, HISL-19, VGF and proSAAS). Focus on molecular and biochemical aspects and the functional biological role of the major derived peptides
Montero-Hadjadje M, Vaingankar S, Elias S, Tostivint H, Mahata SK, Anouar Y	Chromogranins A and B and Secretogranin II: Evolutionary and Functional Aspects	45	2008	Focus on CgA, CgB and SgII: evolution, chemical properties and functional role as propeptide precursors and granulogenic factors
Zhao E, Zhang D, Basak A, Trudeau VL	New Insights into Granin-Derived Peptides: Evolution and Endocrine Roles	372	2009	Critical evaluation of the evolution of granin protein; focus on distribution and function of vasostatin, CgB1–41 and secretoneurin
Bartolomucci A, Pasinetti GM, Salton SR	Granins as Disease-Biomarkers: Translational Potential for Psychiatric and Neurological Disorders	293	2010	The biomarker potential and functional role in neurological and psychiatric disorders of CgA, CgB, SgII, SgIII, HISL-19, 7B2, NESP55, VGF and proSAAS fragments/peptides
Conlon MJ	Granin-Derived Peptides as Diagnostic and Prognostic Markers for Endocrine Tumors	290	2010	Focus on CgA, CgB, and SgII marker for endocrine tumors
Portela-Gomes GM, Grimelius L, Wilander E, Stridsberg M	Granins and Granin-Related Peptides in Neuroendocrine Tumours	295	2010	Focus on CgA, CgB, SgII, SgIII, HISL-19, 7B2, NESP55, VGF, and proSAAS marker for neuroendocrine tumors
Proceedings/special issues on the granin proteins				
Helle KB, Aunis D	Chromogranins: Functional and Clinical Aspects	373	2000	Proceedings of Session VII of the 10th International Symposium on Chromaffin Cell Biology; published in the book series Advances in Experimental Medicine and Biology, Vol. 482
Hernández-Cruz A, Eiden LE (eds)	The Chromaffin Cell as a Stress Transducer	430	2010	Proceedings of the 15th International Symposium on Chromaffin Cell Biology published in Cellular and Molecular Neurobiology, issue 30 (8), 2010. (Cited Refs. include 140, 141, 310, 374)
Vaudry H, Metz-Boutigue M-H (eds)	GRANINS: Thirty-Five Happy Years in the Granulosome World	431	2010	Special issue published in Regulatory Peptide, 165 (1), 2011. (Cited Refs. include 238, 239, 257, 290, 295, 311, 375–385)

The *top section* shows results of an ISI search conducted on March 14, 2011, using granin, chromogranin, secretogranin, VGF, proSAAS, or NESP-55 as topic search criteria appearing in title and/or abstract. Additional reviews covering the granin family, and those included in three special issues/proceedings, are also noted.

the study of soluble proteins that were released from the adrenal medulla or were constituents of catecholamine-containing CG, obtained by subcellular fractionation (34–39). The most abundant protein initially identified in adrenal and later parathyroid CG (40) was CgA, representing almost 50% of the soluble protein content of the

TABLE 2. Comparison of granin proteins

Granin	Preprotein AA[h]	Preprotein Calculated MM[h] (kDa)	Mature protein AA[h]	Mature protein Calculated MM[h] (kDa)	Mature protein Observed MM (kDa)	Dibasic sites[h]	AA/% proline[h]	AA/% glutamate[h]	pI calc[h]/obs	% α-Helix[h]
CgA	457	51	439	49	75[h]	10	29/6.3	90/19.7	4.5/4.9[h]	38
CgB	677	78	657	77	110[h]	16	34/5.0	116/17.1	4.8/5.2[h]	26
SgII	617	68	587	68	86[b]	9	42/6.8	78/12.6	4.5/5.0[b]	40
SgIII (i1)	468	53	449	51	57[m]	6	20/4.3	55/11.8	4.8/5.1[m]	46
7B2 (i1)	212	24	186	21	21[p]	4	20/9.4	15/7.1	6.1/5.0[p]	30
NESP55	245	28	201	23	28[m]	9	27/11.0	37/15.1	4.7/5.0[m]	25
VGF	615	67	593	65	90[h]	10	77/12.5	97/15.8	4.5/ND	39
ProSAAS	260	27	227	24	27[r]	6	34/13.1	18/6.9	5.5/ND	42

Number of amino acids (AA) and calculated molecular mass (MM) of the preprotein, number of amino acids and calculated molecular mass of the mature protein, observed molecular mass of the mature protein, number of dibasic sites, number/content of proline, number/content of glutamate, calculated (calc) and observed (obs) pI, and secondary structure (percent α-helix) predicted using PSIPRED (386, 387) are shown for human (h), bovine (b), porcine (p), rat (r), or mouse (m) granin proteins. i1, Isoform 1; ND, not determined.

adrenal chromaffin secretory granule (41). Approximately 20 yr after the initial description of CgA (11), an immunologically and structurally related protein was identified in the CG and in brain, CgB (41), and in subsequent years, additional similar proteins have been discovered, biochemically characterized, and cloned (*e.g.*, SgII) (42). The chromogranin and secretogranin proteins share many properties, including acidic isoelectric point (pI), binding to calcium, propensity to form aggregates, and the presence of multiple dibasic cleavage sites, all of which are discussed in *Section II* and have been described in several excellent reviews (11, 43–45). Indeed the presence of chromogranins in secretory vesicles, coupled with their high capacity for Ca^{2+} binding, are critical for Ca^{2+} storage and provide a sizeable intracellular reservoir of Ca^{2+} that can be mobilized via inositol 1,4,5-triphosphate (IP3)-receptor (IP3R)/Ca^{2+} channels (46, 47). Subsequent studies indicate that the extended granin family is substantially larger than the chromogranins, CgA and CgB, and secretogranins, SgII and SgIII, and now includes HISL-19 antigen (SgIV), 7B2 (SgV), neuroendocrine secretory protein of Mr 55,000 (NESP55) (SgVI), VGF (SgVII), and proSAAS (SgVIII) (1). The majority of these proteins are the precursors of biologically active peptides, modulating, for example, pain pathways, inflammatory responses, metabolic and mood disorders, and blood pressure (BP). Chromogranin-derived peptides have been previously reviewed (48–50) and are updated here in *Section IV* where additional peptides derived from the extended granin family are described.

Analysis of the genomic structural organization and coding sequences of individual granin proteins suggest functional conservation throughout vertebrate evolution (45, 51). Furthermore, recent single-nucleotide polymorphism (SNP) characterization of the human *CHGA* (CgA) (52) and *SCG3* (SgIII) (53) genes is consistent with an important functional contribution of these granins to hypertension and obesity, respectively (see *Section V*). Moreover, the relative abundance of granins is likely responsible for their expanding utility as disease biomarkers (see *Section VI*).

II. Structural Comparison of Granins

Biochemical and structural features of the granin proteins CgA, CgB, SgII, SgIII, 7B2 (SgV), NESP55 (SgVI), VGF (SgVII), and proSAAS (SgVIII)[1] are reviewed below and summarized in Table 2. In addition, evolutionary conservation is discussed, drawing on protein sequence data from vertebrates (*e.g.*, zebrafish to human) and invertebrates.

A. Why consider the granins as members of a structurally and functionally related family?

Granins are relatively abundant, acidic proteins that are localized in secretory vesicles, where they bind to calcium, aggregate, and share a number of biochemical features that are summarized in Table 2 (see also Refs. 43 and 44). What differentiates granins from classical peptide precursors that are also found in the secretory pathway? Classification is not absolute, but generally protein size and pI do provide some guidance. The largest known classical mammalian neuropeptide precursors have a size of approximately 30 kDa [proopiomelanocortin (POMC) and proenkephalin; 267 amino acids)], whereas the remainder are smaller, usually in the 10- to 15-kDa range. The granin proteins reviewed here are all larger than 24 kDa, with the majority sized greater than 50 kDa (see Table 2). The pI values for these granins range from 4.5–6.1 with a mean of 4.9, whereas neuropeptide precursors generally have higher pI (5.1–11.4) with a mean of 7.1

[1] Throughout the text, we refer to the granin proteins by their published names. However, we also note here the secretogranin nomenclature (SgX) introduced by Helle in 2004 (44) that conveys the notion that granin proteins are structurally and functionally related.

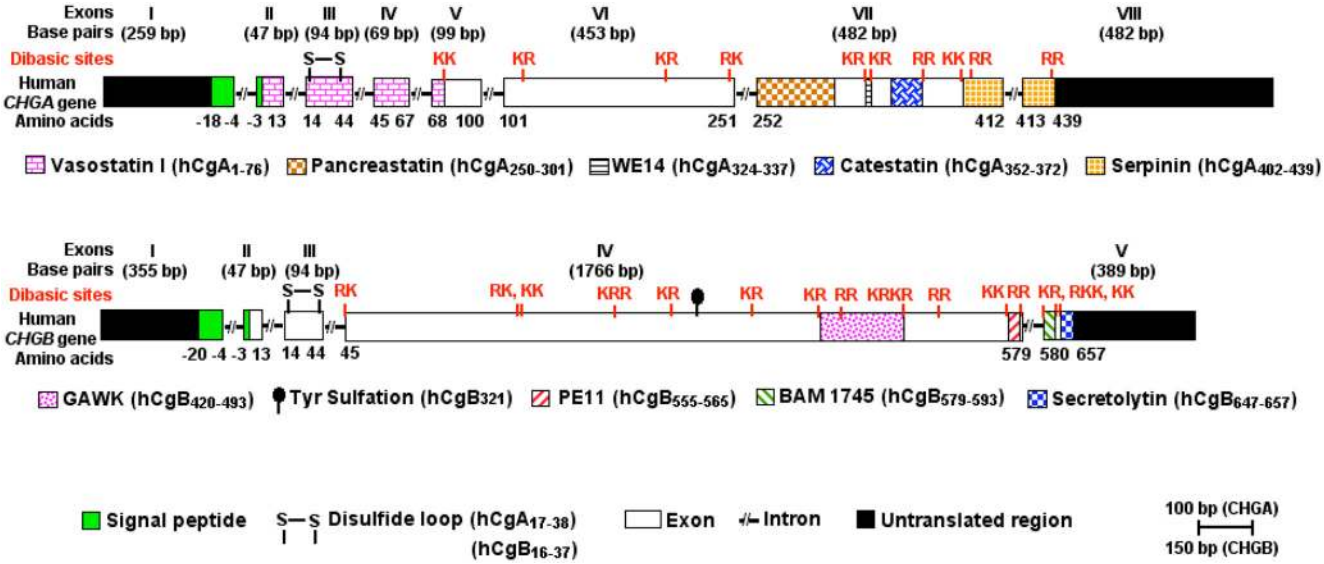

FIG 1. Genomic organization of human CgA and CgB. Positions of sequences encoding conserved, biologically active CgA- and CgB-derived peptides within the human *CHGA* and *CHGB* genes, respectively, are shown.

(n = 15).[2] Although granins and peptide precursors have multiple dibasic cleavage sites, and both groups undergo differential cell-type-specific and tissue-specific processing, cleavage of classical neuropeptide precursors at specific dibasic residues to mature peptides is usually more easily predicted and complete (20, 54–56) and in neurons may even occur locally in axon terminals and dendrites (57). Perhaps the relative resistance of granins to complete cleavage is a function of secondary structure (54, 58) and/or state of aggregation. Interestingly, calcium binding at low pH and intermolecular aggregation have been noted rarely for peptide precursors (*e.g.*, protachykinin) (59) but are very commonly associated with granins, where they play a role in sorting into LDCV and the regulated secretory pathway. Can granins be easily differentiated from the precursors of growth factors, cytokines, and hormones that are also localized in the secretory pathway? These proteins also tend to be smaller when compared with granins (10–40 kDa) and can bind divalent cations, and although many are processed from proforms, they are rarely cleaved at multiple paired basic residues into peptides, the hallmark of granins. So although there are no absolute guidelines that define granin proteins, there are advantages to discussing these eight highly similar proteins as an extended family.

[2] Mean pI was calculated from the following human mature neuropeptide precursors: agout-related protein, cocaine- and amphetamine-regulated transcript, cholecystokinin, galanin, ghrelin, GnRH, neurotensin, neuromedin U, neuropeptide W, neuropeptide Y, POMC, proenkephalin-A, protachykin β, somatostatin, and vasoactive intestinal polypeptide.

B. The original granin proteins: CgA and CgB

1. Chromogranin A

The human *CHGA* (CgA) gene is located on chromosome 14q32.12, which spans 12,192 bp and gives rise to a transcript of 2,041 bp that encodes a 439-amino-acid mature protein (9). There are 10 dibasic sites in human CgA, which are potential sites for proteolytic cleavage (60). The dibasic sites in CgA from other species range from a minimum of seven in mouse to a maximum of 11 in African clawed frog (61–70). In homeothermic vertebrates, CgA is an approximately 48- to 52-kDa protein with a coiled-coil structure (71). The *chga* genomic organization has been reported for bovine (72), human (73), and mouse (66). The human *CHGA* gene is organized in eight exons and seven introns (Fig. 1). Exon I encodes the 5′-untranslated region (UTR) (260 bp) of the CgA mRNA and most of the signal peptide of CgA. Exons II–V encode the vasorelaxant and cardiosuppressive peptide vasostatin (VST: $hCgA_{1–76}$) (74). VST is highly conserved across vertebrates: human *vs.* mouse, approximately 87%; human *vs.* chicken, approximately 79%; human *vs.* marsh frog, approximately 71%; and human *vs.* zebrafish, approximately 59%. Exon III encodes highly conserved cysteine residues that form the disulfide loop of CgA. Exon VI contains the most variable peptide sequences across species. Exon VII encodes most of the biologically active peptides including dysglycemic hormone pancreastatin (PST: $hCgA_{250–301}$) (75), catecholamine release-inhibitory and antihypertensive peptide catestatin (CST: $hCgA_{352–372}$) (76), and 14 amino acid peptide with N-terminal tryptophan (W) and C-terminal glutamatic acid (E) (WE14) ($hCgA_{324–337}$), which acts as an autoantigen in type 1 di-

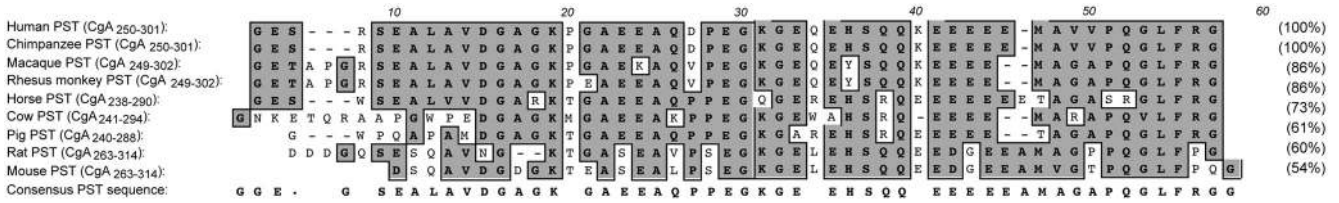

FIG. 2. Alignment of the PST domain of CgA. PST alignment in mammalian species was performed using the ClustalW program of MacVector version 9.0, and the percentages of homology were calculated (shown in *parentheses*). The most conserved amino acids are *highlighted in gray*. PST sequences used are human (accession number NM_001275), chimpanzee (XM_510135), macaque (AB_169793), rhesus monkey (XM_001092629), horse (NM_001081814), cow (NM_181005), pig (NM_001164005), rat (NM_021655), and mouse (NM_007693).

abetes (77). Among CgA peptides, PST is the least conserved, having only 54% homology between human and mouse (PST homology cannot be ascertained in nonmammalian vertebrates) (Fig. 2). Unlike PST, CST is highly conserved in mammals, with approximately 86% homology between human and mouse. Human CST bears moderate homology with nonmammalian vertebrates: 38% with jungle fowl, 33% with frog, and approximately 19% with zebrafish (Fig. 3). WE14 is the most conserved CgA peptide in mammals, where it is 100% conserved except for pig (~93%). Like VST, human WE14 is highly conserved with marsh frog (71%), but less conserved with zebrafish (21%). Exon VIII contains the C terminus of the protein, including the last dibasic amino acid pair, and the 3′-UTR (407 bp) of the CgA mRNA. Encoded by part of exons VII and VIII is a highly conserved peptide, serpinin (bCgA $_{403–428}$), that is approximately 90% homologous among human, bovine, and mouse and approximately 70% homologous with zebrafish (78).

2. Chromogranin B

The CgB gene (*CHGB*) located on human chromosome 20pter-p12 comprises five exons (79). The 2666-bp mRNA transcribed from this gene encodes a preproprotein of 677 amino acids containing 16 pairs of consecutive basic amino acids (Fig. 1). CgB shares several features with CgA, including wide expression throughout the endocrine and nervous systems, acidic protein backbone, random-coil structure, and heat stability. Furthermore, two distinct sites, the Cys loop at the N terminus and a C-terminal region, share significant sequence homology (more than 40% sequence identity), and this conservation is extended to nonmammalian vertebrates (80). CgB is abundantly expressed in many neurons and peptidergic endocrine cells (9, 43, 48). After synthesis, CgB is posttranslationally O-glycosylated and sorted to large secretory vesicles. CgA and CgB represent the predominant proteins found in adrenal CG. The relative concentrations, however, vary between species; in bovine CG, more CgA than CgB is present, whereas in human and rats, more CgB than CgA is synthesized (81). Within granules, CgB is proteolytically processed at dibasic LysArg and monobasic Arg sites to several proteins of intermediate size and small peptides (82). Three small peptides, BAM-1745 (83), PE11 (84), and secretolytin (85), were characterized further. Only secretolytin, a peptide that is conserved in a number of species, was found to have biological activity as an antibacterial agent.

C. Additional members of the granin family: SgII, SgIII, 7B2, NESP55, VGF, and proSAAS

1. Secretogranin II

The SgII gene (*SCG2*), located on human chromosome 2q35-2q36 (79), comprises two exons. Exon 1 encodes

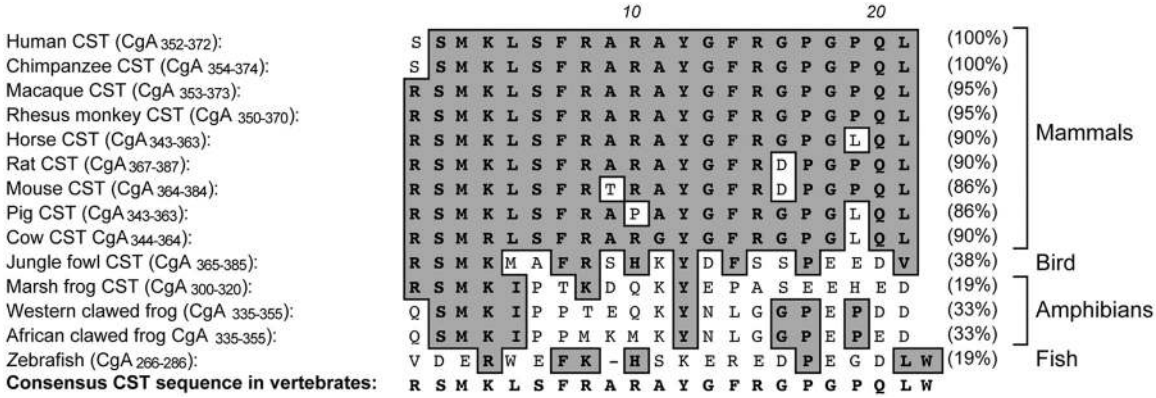

FIG. 3. Alignment of the CST domain of CgA. CST alignment in vertebrate species was performed using the ClustalW program of MacVector version 9.0, and the percentages of homology were calculated (shown in *parentheses*). The most conserved amino acids are *highlighted in gray*. CST sequences used are human (accession number NM_001275), chimpanzee (XM_510135), macaque (AB_169793), rhesus monkey (XM_001092629), horse (NM_001081814), rat (NM_021655), mouse (NM_007693), pig (NM_001164005), cow (NM_181005), jungle fowl (XM_421330), and marsh frog (AF139924).

215 nucleotides of the 5′-UTR, and exon 2 encodes 14 nucleotides of the 5′-UTR plus the entire coding region and 3′-UTR of SgII. SgII is a 617-amino-acid preproprotein with nine pairs of consecutive basic amino acids. Endoproteolytic processing at these sites generates intermediate-sized proteins as well as several small peptides, secretoneurin (SN) (86, 87), EM66 (88), and manserin (89). The degree of processing is variable, generally higher in the nervous system although less pronounced in the adrenal medulla, where the high concentration of catecholamines significantly inhibits proteolytic enzymes (90). SN is highly conserved across evolution; SN is 90–100% identical between mammals and 84–87% identical between human and cartilage fish (91, 92). Bony fish SN is 45–67% identical to mammalian SN; in some bony fish, two SN variants with differing N-terminal sequences are found in the genome, resulting from ancestral gene duplication. In addition to SN, EM66 is another highly conserved peptide within SgII (50–70% identical between human and lower vertebrates) (93–96).

2. Secretogranin III

The SgIII gene (*SCG3*) located on human chromosome 15q21 comprises 12 exons. The 3366-bp SgIII mRNA encodes a 468-amino-acid acidic secretory protein with seven pairs of consecutive basic amino acids that is well conserved during evolution, from mammals to fish (Fig. 4A). SgIII is synthesized as an N-glycosylated protein and cleaved proteolytically in secretory vesicles to intermediate-sized proteins (97). No biologically active peptides derived from SgIII have been described.

3. 7B2 gene and protein

Human 7B2 (*SCG5*), located on chromosome 15q13-q14, has six exons. Two variant mRNA of 1244 and 1241 bp are transcribed, the longer encoding a protein that is one amino acid longer than that encoded by the shorter transcript, which uses an alternate in-frame splice junction. 7B2 and proSAAS, which is discussed in *Section II.C.6*, have the least acidic pI of the granin proteins reviewed here and exhibit functional and structural homology to one another, with each containing a C-terminal peptide inhibitor of PC catalytic activity (98, 99). 7B2 is perhaps the most evolutionarily conserved member of the granin family, particularly in vertebrates but also with orthologs identified in several invertebrate species including *Aplysia*, *Caenorhabditis elegans*, and *Drosophila* (Fig. 4B). Two highly conserved regions stand out, a proline-rich sequence that is critical for 7B2 function as a chaperone of PC2, the other encompassing the C-terminal peptide inhibitor of PC2 catalytic activity (reviewed in Ref. 100). Comparison of PC2 and 7B2 patterns of expression, the former a subset of the latter, and the phenotypes of 7B2- and PC2-null mice, the former developing a lethal form of Cushing's disease whereas the latter remain generally healthy, suggests that 7B2 may chaperone other proteins in addition to PC2 (101, 102).

4. NESP55 gene and protein

NESP55 is part of the extremely complex imprinted *GNAS* gene locus on human chromosome 20q13.2, which encodes the α-subunit of the stimulatory G protein (Gsα). By using multiple promoters and different first exons, mRNA encoding several distinct proteins including Gsα, NESP55, XLas, and 1A, and also antisense transcripts (termed nespas in mice), are transcribed from this locus (103–106). The *NESP55* exon encoding the 5′-untranslated RNA plus the complete open reading frame of NESP55 is spliced onto exons 2–13 of Gsα (107). This splice pattern is found in all species analyzed so far. In addition, further splicing in the 3′-untranslated RNA leads to one prominent shorter mRNA variant (108). Any of these NESP mRNA can be genomically imprinted and transcribed only from the maternal allele. The NESP55 protein consists of 244 amino acids and has six pairs of consecutive basic amino acids in its primary sequence. At these sites, NESP55 is cleaved to smaller peptides including the C-terminal peptide GAIPIRRH (107).

5. VGF gene and protein

The human *VGF* gene located on 7q22.1 is comprised of two exons. The 2586-bp transcript encodes a 615-amino-acid protein, with the entire protein-coding sequence found uninterrupted on exon 2 of the *VGF* gene. As shown in Table 2, VGF is an acidic, proline- and glycine-rich polypeptide. Mammalian VGF orthologs have 10–11 conserved clusters of basic residues, many of which are processed in endocrine, neuroendocrine, and neuronal cells in a tissue-specific manner, generating a number of peptide fragments (109). Comparison of mammalian VGF proteins reveals several regions of high sequence conservation that are shared by a putative zebrafish VGF ortholog (Fig. 4C), whereas no closely related invertebrate VGF proteins have been identified. Sequence conservation in the zebrafish VGF ortholog may define structurally or functionally important regions of the protein; the neuroendocrine regulatory peptide (NERP) and C-terminal peptide regions that encode a number of bioactive peptides (see *Section IV*) are the most highly conserved (Fig. 4C).

6. ProSAAS gene and protein

The human *PROSAAS* (*PCSK1N*) gene is found on chromosome Xp11.23 and includes three exons. The 990-bp proSAAS transcript encodes a 260-amino acid precursor protein. Analogous to 7B2, the most highly conserved protein segment within proSAAS, approximately

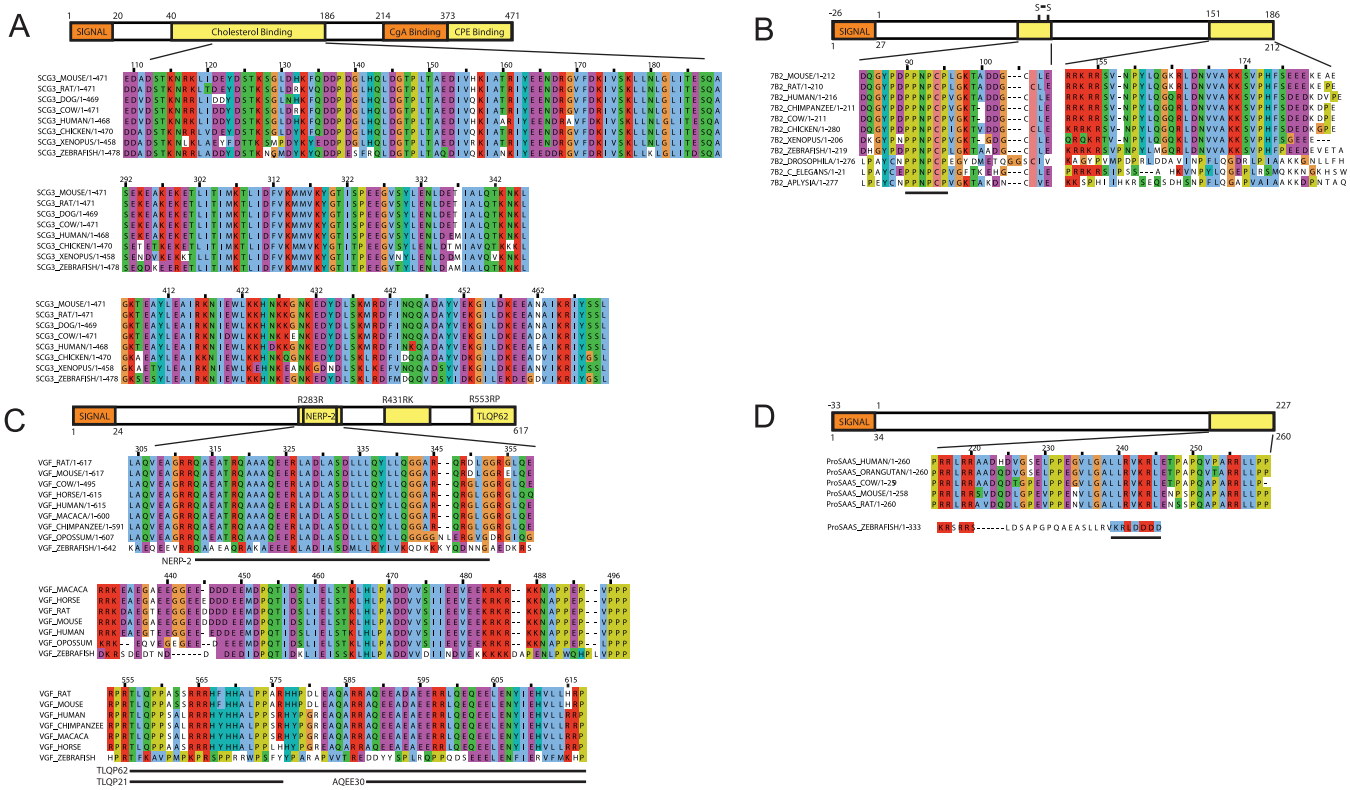

FIG. 4. Evolutionary conservation of vertebrate and invertebrate granins. Protein sequences for SgIII (SCG3), 7B2, VGF, and proSAAS were aligned using the program ClustalW2 (422), edited with Jalview version 2.5.1 (423), and displayed using the ClustalX color scheme. Amino acid numbers shown in the alignments correspond to the entire protein coding sequences for rat SCG3 (A), the mature human 7B2 protein (B), rat VGF (C), and human proSAAS (D). Three functional domains of SCG3, which bind to cholesterol, CgA, and carboxypeptidase E (CPE), each contain regions that have been highly conserved throughout vertebrate evolution (A). For 7B2, the most highly conserved regions include the cysteine-stabilized PC2 interaction domain that is conserved even in invertebrates, and the C-terminal peptide catalytic inhibitor of PC2, which is highly conserved in vertebrates (B). Three highly conserved regions of VGF, one that includes the NERP-2 peptide and another that includes the C-terminal TLQP and AQEE peptides, are each conserved in higher and lower vertebrates (C). For proSAAS, the C-terminal PC1/3 inhibitory peptide with its LLRVKRL motif is conserved in vertebrates (D). No bird orthologs of VGF or proSAAS were identified using looser BLAST search parameters (51), querying with these short conserved domains. SgIII (SCG3) sequences are human isoform 1 (NP_037375), dog (XP_535482), cow (NP_001095567), mouse (NP_0331561), rat (NP_446308), chicken (XP_413807), *Xenopus* (NP_001079046), and zebrafish (NP_957051). 7B2 sequences aligned are human isoform I (accession number NP_001138229), mouse (NP_033188), rat (NP_037307), cow (NP_001039463), chimpanzee (NP_001092019), zebrafish (NP_957020), *Aplysia* (ABF21075), *C. elegans* (NP_508020), and *Drosophila* isoform B (NP_001014608). VGF sequences aligned are human (NP_003369), rat (NP_112259), mouse (NP_001034474), cow (XP_875466), horse (XP_001916046), macaca (NC_007860), chimpanzee (XP_519275), opossum (XP_001371271), and zebrafish (XP_001343121). ProSAAS sequences included are human (NP_037403), mouse (NP_038920), rat (NP_062152), cow (NP_001077149), orangutan (XP_002831656), and zebrafish (NP_001159601).

85% identical between zebrafish and mouse, is part of the C-terminal peptide inhibitor of PC1/3 catalytic activity (ELLRVKRL; conserved sequence is *underlined*) (Fig. 4D). Although the processed peptides from proSAAS are conserved in higher vertebrates, this does not generally extend to lower vertebrates, suggesting that proSAAS may function as a peptide precursor only in higher vertebrates, although its endocrine and neural distribution is conserved in *Xenopus* and *Danio rerio* (51).

III. Sorting and Granulogenesis

A. Biosynthesis and intracellular trafficking of granins

Granins are expressed in endocrine cells and peptidergic neurons, which in addition to having the constitutive secretory pathway that is present in all cells also have a regulated secretory pathway (110). Proteins are secreted through the constitutive pathway continuously, whereas secretion through the regulated pathway occurs only when the cell is stimulated by a secretagogue. Granins are synthesized at the rough endoplasmic reticulum (RER) and inserted into the RER cisternae via the signal peptide located at the N terminus of the molecule. They are subsequently transported from the RER to the Golgi complex via transport vesicles similar to other secretory proteins (111). Within the Golgi stacks, the granins are sorted away from constitutively secreted and lysosomal proteins. They are then packaged at the TGN into immature granules together with other granule proteins such as prohormones and their processing enzymes. Granins are partially pro-

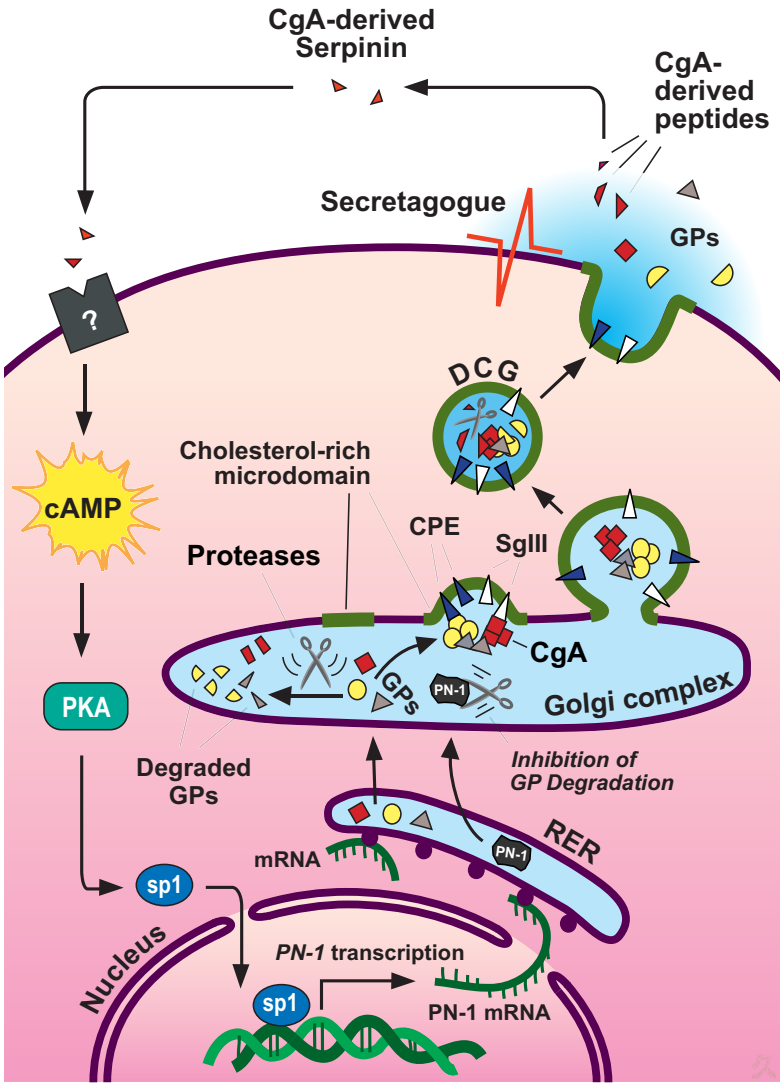

FIG. 5. Model for intracellular trafficking of granule proteins and autocrine regulation of DCG biogenesis by the CgA-derived peptide serpinin in endocrine cells. Granule proteins (GPs, including granins and prohormones) are synthesized at the RER and then transported to the Golgi complex where they are sorted at the TGN into the regulated secretory pathway. Granins (*e.g.*, CgA) and prohormones form aggregates, which bind to SgIII or carboxypeptidase E (CPE), sorting receptors that are anchored to cholesterol-sphingolipid-rich membrane microdomains at the TGN. These membrane domains bud under the driving force of the granin (CgA and CgB) aggregates to form immature granules. Specific proteolytic enzymes process the prohormones fully or the granins partially to yield biologically active peptides. The granins, the major protein in the granules, condense to form mature DCG. Excess granule proteins are degraded in the Golgi complex. Upon stimulation of the cell, DCG exocytose and release their contents. In cells expressing CgA, a C-terminal peptide, serpinin, is released and binds to a putative G protein-coupled receptor to increase the transcription and biosynthesis of protease nexin-1 (PN-1) via a cAMP-PKA-Sp1 signal transduction pathway. PN-1 inhibits GP degradation to increase GP levels, which in turn leads to more DCG formation to replenish the ones secreted.

cessed to various biologically active peptides in the immature secretory granules, which then undergo maturation involving further acidification of the granule milieu and removal of the clathrin coat and synaptotagmin IV and vesicle-associated membrane protein 4 from the granule membrane (reviewed in Ref. 112). Mature secretory granules are stored, and their contents are released upon stimulation (Fig. 5).

B. Mechanisms of granin sorting into regulated secretory pathway granules

Studies on the sorting of granins into regulated secretory pathway granules have focused mainly on CgA, CgB, and SgIII, although some work has also been done on SgII and VGF but not on the other granins. Initial investigations have proposed that because granins are highly acidic proteins and tend to aggregate at low pH in the presence of calcium in the TGN, they are sorted out from other proteins and enter the budding granules in a passive manner (113, 114). However, later studies have identified sorting determinants on the granins, suggesting that active interaction with a specific binding protein (*i.e.*, sorting receptor) or a lipid may be involved in the sorting mechanism (as detailed in *Section III.B*) (115–124). Furthermore, a number of studies have indicated that aggregation alone is not sufficient for sorting of CgA, CgB, and VGF (117, 120). Nevertheless, aggregation remains an important first step for concentrating the granins at the TGN before binding to membrane components for efficient sorting. Targeting signals for CgA, CgB, SgIII, SgII, and VGF (Fig. 6) have been reported, along with the interacting membrane receptors for CgA and SgIII. These are described in the following sections.

1. Chromogranin A

Taupenot's group (121, 125) has shown that the N-terminal domain of CgA (bovine/human residues 40–115) not containing the disulfide bonded loop structure (Cys17-Cys38) is necessary for directing CgA into the secretory granules of PC12 cells. Additionally, Hosaka *et al.* (119) have shown that the N-terminal domain (rat/bovine residues 48–111/48–95) of CgA was essential for binding to SgIII, the proposed sorting receptor for CgA. This domain of CgA binds strongly to a SgIII domain comprising residues 214–373 at pH 5.5 in the presence of 10 nM calcium. SgIII itself is anchored to cholesterol-rich membranes in secretory granules, which are derived from the TGN. These investigators also demonstrated that the CgA-SgIII interaction was necessary for targeting CgA to granules of the regu-

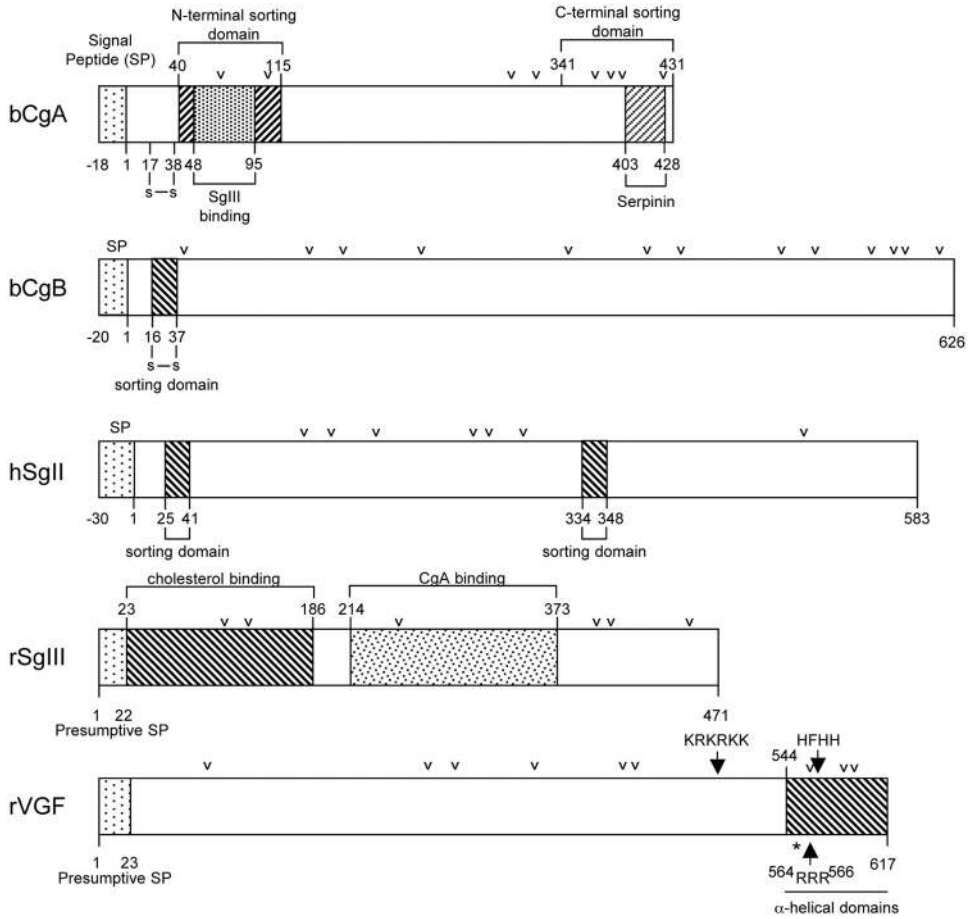

FIG. 6. Schematic diagram showing the structures and sorting domains of bovine (b) CgA, bovine CgB, human (h) SgII, rat (r) SgIII, and rat VGF. The sorting determinants of these granins and the binding sites referred to in the text are indicated. The *arrowheads* represent paired or multiple basic residues that are potential PC cleavage sites. The *asterisk* in the VGF structure represents a noncanonical cleavage site ^{553}RPR^{555} that is cleaved to generate a number of peptides.

lated secretory pathway in AtT-20 cells, a pituitary cell line; PC12 cells, a neuroendocrine cell line; and pancreatic β-cells (119, 123). However, Cowley *et al.* (126) showed that a 90-amino acid evolutionarily conserved C-terminal domain, not the N-terminal domain, was critical for sorting CgA to the regulated secretory pathway in GH4C1 cells but played no role in PC12 cell sorting. It is unknown whether this C-terminal domain binds SgIII or other membrane components. In another study, transfection of N- or C-terminal truncated frog CgA into COS-7 cells resulted in retention of these mutant forms in the Golgi complex, whereas the full-length form induced secretory granule biogenesis (124). Hence, both the N and C termini may have targeting information, but there is cell specificity in terms of which sorting determinant is used to sort CgA (11, 116, 119, 124–129).

2. Chromogranin B

Gerdes and co-workers (115) have identified an N-terminal disulfide bonded loop within the first 37 amino acids of CgB (Fig. 6) that was essential for sorting this granin into the regulated secretory pathway in PC12 cells. They found that this signal, when fused to an α1-antitrypsin reporter, a constitutively secreted protein, was sufficient to direct this protein to the regulated secretory pathway (130). More importantly, this signal functions at the level of the TGN by binding to membrane components that give rise to secretory granules. However, the membrane components have not been identified. Interestingly, the sorting efficiency of the α1-antitrypsin reporter protein was increased 5-fold when two loops were present. Multiples of these loop-sorting determinants present on the surface of aggregates of CgB could lead to enhanced binding to membranes, thereby increasing sorting efficiency at the TGN. However, this loop structure is not required for sorting CgB into the regulated secretory pathway in GH4C1 cells, again showing cell-type specificity in the sorting determinants used.

3. Secretogranin II

Truncation analyses of SgII revealed targeting signals in both the C and N termini. Courel *et al.* (122) found that two putative α-helix-containing domains, $hSgII_{25-41}$ and $hSgII_{334-348}$, can act independently, and each is sufficient

for sorting SgII into regulated secretory pathway granules in PC12 cells. However, it is unclear whether these sorting domains interact with membrane components at the TGN to mediate sorting. Interestingly, it has been shown in a yeast two-hybrid system that SgII interacts with SgIII (131). This raises the possibility that in cells such as those in the sympathoadrenal system that contain both SgII and SgIII, SgII could be targeted to the secretory granules by binding to TGN membrane-anchored SgIII.

4. Secretogranin III

SgIII is sorted into secretory granules by binding to cholesterol-rich membranes at the TGN in AtT-20 cells (132). Structure-function analysis has identified an N-terminal domain comprised of residues 23–186 (rat) of SgIII that specifically binds to cholesterol (132). Cholesterol, an integral part of the TGN membrane, plays a critical role in curvature formation for granule biogenesis (112, 133). Thus, SgIII, by binding to cholesterol in TGN membrane domains, which are destined to be budded off to become the secretory granule membrane, facilitates its own targeting to the regulated secretory pathway. Concomitantly, SgIII anchored to these cholesterol-rich domains in the TGN membrane is poised to act as receptor for sorting CgA and other prohormones into the regulated secretory pathway (33).

5. VGF sorting

Studies on VGF sorting have identified a C-terminal 73-amino acid fragment (rat 545–617) containing two predicted α-helix domains and four PC cleavage sites (Fig. 6), which was sufficient for targeting this granin to the regulated secretory pathway in PC12 and INS cells (120). Mutation studies indicate that although the helical domains are not necessary, the ^{564}RRR^{566} PC cleavage site and adjacent HFHH domain, and PC catalytic activity, each contribute to VGF sorting and release. As yet another example of cell type-specific differences in sorting, expression of VGF and multiple deletion mutants in rat FRT thyroid cells resulted, in all cases, in regulated secretion from apical domain LDCV (134).

C. Function of granins in dense core secretory granule biogenesis

Several granins, including CgA, CgB, SgII, and SgIII, have been demonstrated to be granulogenic proteins. CgA or CgB when overexpressed in fibroblasts induced granule-like structures with a dense core, which were capable of releasing their contents in a regulated manner (30, 31, 135). Down-regulation of CgA expression in PC12 cells or in a transgenic mouse by antisense CgA resulted in decreased DCG biogenesis in chromaffin cells (136). Additionally, a CgA knockout (KO) mouse (CgA-KO) exhibited decreased DCG in the adrenal medulla (137). On the other hand, Hendy *et al.* (138) reported normal DCG biogenesis in the adrenal medulla of the CgA-KO they generated, but other granin levels were increased, which could have compensated for the lack of CgA. Likewise, a CgB-KO showed normal DCG biogenesis and morphology due to compensatory granin biosynthesis (139). Depletion of SgII expression by SgII small interfering RNA in PC12 cells led to a decrease in both number and size of DCG (32). These *in vivo* and *in vitro* studies taken together support the function of granins in DCG formation.

Granins aggregate into large complexes at low pH in the presence of calcium at the TGN (113). A granulogenic N-terminal determinant in CgA, located within b/hCgA$_{40–115}$ of the mature protein (Fig. 6), has been shown to facilitate aggregation. These granin complexes then interact directly or indirectly with cholesterol-sphingolipid-rich membranes, providing the driving force to induce budding at the TGN to form DCG. For a review on membrane lipids involved in granule biogenesis, see Kim *et al.* (112). Because SgIII is anchored to cholesterol-rich domains in the TGN membrane, it could facilitate DCG biogenesis by providing a platform for recruitment of the granin complexes (Fig. 5). Within the DCG, a coiled-coil structure of CgA has been suggested to play a role in granule core condensation (71). Thus, granin proteins function in granule biogenesis and the sorting and secretion of other proteins including peptide hormones from the regulated secretory pathway, as further detailed in several excellent recent reviews (78, 140, 141).

D. Regulation of DCG biogenesis by the CgA-derived peptide serpinin

Initial studies by Kim *et al.* (30, 142) demonstrated that CgA, but not CgB, played an important role in regulating DCG biogenesis in PC12 cells and pituitary 6T3 cells, a mutant cell line of AtT-20 cells that lacks DCG. Recent studies in pituitary AtT-20 cells have provided evidence for an autocrine mechanism that up-regulates DCG biogenesis to replenish these granules after stimulated exocytosis (Fig. 5). The autocrine signal was identified as serpinin, a novel 26-amino acid peptide that is cleaved from the C-terminal region of CgA (Fig. 6) (78). Serpinin was first isolated from AtT-20 cell-conditioned medium and is released in an activity-dependent manner from DCG. Subsequently, serpinin was found to increase cAMP levels in the cell, presumably through binding to a putative G protein-coupled receptor and activation of adenyl cyclase. This then led to an increase in transcription of a protease inhibitor, protease nexin 1 (PN-1). A protein kinase A (PKA) inhibitor blocked the increase in PN-1 mRNA in serpinin-treated AtT-20 cells. PN-1 was shown to inhibit

degradation of granule proteins, including granins in the Golgi complex, stabilizing those proteins and increasing their levels, which then significantly enhanced DCG formation and granule numbers (78, 112). The up-regulation of transcription of PN-1 mRNA was found to be mediated by the transcription factor Sp1, which upon serpinin treatment of the AtT-20 cells, translocated from the cytoplasm to the nucleus. The PN-1 promoter contains several Sp1-binding sites, and mithramycin A, an inhibitor of Sp1 binding to DNA, blocked the up-regulation of PN-1 transcription in the presence of serpinin. Additionally, a luciferase-reporter assay demonstrated that mutation of the Sp1 promoter inhibited serpinin-induced up-regulation of PN-1 (143). Thus, as shown in the model developed from studies of AtT-20 cells (Fig. 5), serpinin acts in an autocrine/paracrine fashion to enhance granule biogenesis by up-regulating PN-1 transcription via a cAMP-PKA-Sp1-mediated pathway. Treatment of PC12 cells with serpinin also led to an increase in PN-1 transcription, suggesting that this mechanism of regulation of DCG biogenesis may also extend to other (neuro)endocrine cells (78). Such a mechanism might seem wasteful, but regulation at the post-translational level may in fact be quite efficient to rapidly replenish small numbers of DCG released at any one time, because it is possible to increase the levels of many granule proteins through one step, inhibition of degradation. Interestingly, biogenesis of insulin DCG in pancreatic β-cells is regulated in an autocrine manner involving a posttranscriptional mechanism to transiently stabilize mRNA encoding granule proteins upon glucose stimulation to release insulin (144). Thus, it would appear that autocrine/paracrine signaling to regulate DCG biogenesis at the post-transcriptional/translational level may be used by various endocrine cells to replenish released granules.

E. Regulation of intracellular calcium stores by granin proteins in DCG

Endocrine, neuroendocrine, and neuronal cells secrete a variety of peptides and hormones via calcium-dependent release. The number of DCG in an endocrine cell ($\sim$10,000), and the high Ca^{2+} binding capacity of resident granin proteins and their abundance ($\sim$2–4 mM), together constitute a recently recognized, major intracellular calcium reservoir (46, 47). Coupled with the abundance of IP3R in secretory granule membranes and the direct modulatory interactions demonstrated between either CgA or CgB and IP3R/Ca^{2+} channels, a mechanism has been established for the regulation of cytosolic calcium stores and granule exocytosis in secretory cells. This crucial role of DCG and granin proteins CgA and CgB in calcium homeostasis has been elegantly reviewed elsewhere (46, 47).

IV. Granin-Derived Peptides and Their Mechanisms of Action in Endocrine and Neuroendocrine Systems

Diverse biological activities of specific granin-derived peptides are reviewed below, with greater attention devoted to the endocrine, neuroendocrine, cardiovascular, inflammatory, and neural contributions of peptides derived from CgA, CgB, SgII, 7B2, VGF, and proSAAS. These include roles as PC inhibitors or regulators of PC folding/sorting, and contributions to hormone release (insulin, PTH, and vasopressin), glucose homeostasis, catecholamine release, neuronal excitability, autoimmunity, and smooth muscle and vascular contractility. As a consequence of these activities, granin-derived peptides critically modulate pain pathways, metabolic and mood disorders, and BP. Below, we review the physiological contributions of various granin-derived peptides, organizing the discussion by physiological system, and provide a summary of peptide biological activities, organized by granin (Table 3).

A. Regulation of glucose balance: CgA peptide pancreastatin

The first granin-derived peptide to be discovered was the CgA peptide PST, which was initially identified in porcine pancreas as a C-terminally amidated 49-mer peptide ($pCgA_{240-288}$), which strongly inhibited glucose-induced insulin release from the isolated perfused pancreas (75). Subsequently, PST was found to be a CgA peptide, forming the basis of the prohormone concept for CgA (145). In human plasma, the major form detected was a 52-amino acid PST ($hCgA_{250-301}$). Although it shares five Glu with the gastrin sequence and the carboxyl-terminal sequence Arg-Gly-amide with vasopressin, PST is not homologous to any family of peptides (75) and is found only in mammals, where the homology is relatively low ($\sim$54%) (Fig. 2). After proteolytic cleavage from CgA, PST requires C-terminal amidation by the peptide α-amidating monooxygenase (PAM) for activation. PST exerts multiple, potentially dysglycemic actions, including inhibition of glucose-stimulated insulin release from pancreatic β-cells (75), inhibition of glucose uptake in adipocytes and hepatocytes (146), and induction of glycogenolysis (147, 148). The dysglycemic actions of PST are also achieved in experimental animals *in vivo*. PST stimulates glucagon release *in vivo* in mice (149) and rats (150) and *in vitro* in the pig (151) and inhibits secretion of pancreatic polypeptide (152) and PTH (153) as well as pancreatic exocrine secretion (154). In humans, PST decreases glucose uptake (by $\sim$50%) and increases spillover of free fatty acids (by 4.5- to 6.4-fold) (155). The lack of change in forearm plasma flow indicates a metabolic, rather than hemodynamic, mechanism of action of PST (155). Although PST is elevated in type 2 diabetes mellitus (by $\sim$3.7-fold), it is not

TABLE 3. Biological activities of granins and granin-derived peptides

Peptide (aa mature protein) or mature protein (region)	Biological activities
CgA_H	
VST I (1-76)	Vasodilator, antimicrobial, inhibits PTH secretion, promotes cell adhesion, proapoptotic, inhibits endothelial cell proliferation/migration
VST II (1-115)	Antimicrobial, vasodilator
Chromacin (176-197)	Antimicrobial
PST (250-301)	Inhibits insulin release (β-cells), glucose uptake, PTH release, and glycogenolysis; stimulates glucagon and histamine release
CST (352-372)	Inhibits nAchR and catecholamine release, vasodilator, induces endothelial cell proliferation/migration, reduces cardiac contractility
Serpinin (402-439)	DCG biogenesis; inhibitor of cell death
PST (357-428)	Inhibits PTH release
CgA (1-439)	DCG biogenesis, CgB interactor, SgIII interactor, IP3R interactor; [regulator of DCG biogenesis, BP, blood glucose]
CgB_H	
CgB1-41	Inhibits PTH secretion
Secretolytin (647-657)	Antimicrobial
CgB (1-657)	DCG biogenesis, hormone secretion, catecholamine secretion and vesicle exocytosis, IP3R interactor, CgA interactor; [regulator of DCG biogenesis, BP]
$SgII_R$	
SN (154-186)	Stimulates LH release, stimulates neurotransmitter release (DA, GABA, glutamate), stimulates monocyte and endothelial cell migration
SgII (1-586)	DCG biogenesis
$SgIII_R$	
SgIII (192-351)	CgA interaction domain
SgIII (1-164)	Cholesterol interaction domain
SgIII (351-449)	CPE interaction domain
SgIII (1-449)	DCG biogenesis
$7B2_H$	
C-terminal (CT) (155-185)	PC2 inhibitor
7B2 (1-185; 1-186)	PC2 chaperone; [regulator of PC2 maturation, pituitary hormone secretion]
$NESP55_B$	
LSAL (159-162)	$5\text{-}HT_{1b}$ receptor antagonist
$NESP55_H$ mRNA	NESP55 transcript of complex imprinted *GNAS* gene locus encoding Gsα is involved in pseudohypoparathyroidism
VGF_R	
NERP-1 (262-286)	Suppresses vasopressin secretion
NERP-2 (290-327)	Suppresses vasopressin secretion; stimulates feeding, locomotor activity, body temperature, oxygen consumption
TLQP-62 (533-594)	Increases feeding, antidepressant, increases neuronal electrical excitability, causes mechanical and cold allodynia
TLQP-21 (533-553)	Increases energy expenditure, modulates inflammatory pain and gastric contractility; inhibits feeding in hamsters
AQEE-30 (565-594)	Antidepressant, induces thermal hyperalgesia and penile erection
LQEQ-19 (576-594)	Induces thermal hyperalgesia and penile erection
VGF (1-594)	[Regulator of energy balance, memory, depression, reproduction]
$ProSAAS_R$	
Big PEN-LEN (188-227)	PC1 inhibitor
Little PEN-LEN (188-221)	PC1 inhibitor
ProSAAS (1-227)	PC1 inhibitor; [regulator of prenatal neuropeptide processing, body weight, locomotion]

Biological and biochemical activities of the mature granins and granin-derived peptides are listed. Amino acid (aa) sequence numbering of the individual peptides is based on that of the mature human (H), bovine (B), or rat (R) granin proteins. Please note that the amino acid sequence numbers for VGF, proSAAS, SgIII, and their respective peptides that are shown in this table correspond to the mature proteins, while those referred to elsewhere in this article correspond to the preprotein sequences. Shown in *square brackets* are functions assigned based on the analysis of KO or transgenic mice, so could be due to activities of individual peptides and/or the mature granin. CPE, Carboxypeptidase E; 5-HT, serotonin; GABA, γ-aminobutyric acid; nAchR, nicotinic acetylcholine receptor; DA, dopamine.

significantly elevated in the more modestly insulin-resistant state of obesity and does not change during substantial (~7 kg) weight loss (155), which speaks for pathophysiological changes of PST in diabetes rather than as a response to insulin resistance. Consistent with the antagonistic effect of PST on insulin action, CgA-KO mice display increased insulin sensitivity that is reversed by PST administration (156) (see *Section V.B*). In wild-type mice, plasma PST levels increase with age, indicating that the rise in PST levels parallels reduced insulin tolerance and hence insulin action. These observations establish a role for PST in human intermediary metabolism and disease and suggest that qualitative hereditary alterations in the primary structure of PST may give rise to inter-individual differences in glucose disposition.

B. Regulation of feeding and energy expenditure: VGF NERP and C-terminal peptides

Several lines of evidence demonstrate an important role for VGF and VGF-derived peptides in the regulation of feeding and energy expenditure, stimulated by the initial observation that germline deletion of the *Vgf* gene in mice results in a hypermetabolic, lean, and obesity-resistant phenotype (157–160) (see *Section V.B*). Consistent with these gene KO studies demonstrating VGF function in energy balance, VGF mRNA levels are up-regulated by fasting in the arcuate nucleus (ARC), with leptin treatment limiting the fasting-induced increase (157). Under *ad lib* feeding conditions, VGF colocalizes in the ARC with POMC and modestly with NPY-expressing neurons, which form a critical hypothalamic circuit to regulate food intake and energy expenditure (161), whereas colocalization increases with NPY and decreases with POMC in the fasted state (157). Finally, VGF expression in the ARC nuclei of Siberian hamsters precedes hibernation-induced metabolic and body weight changes (162).

As noted in *Section II*, a number of VGF-derived peptides have been identified, including the peptide designated TLQP-21, which regulates energy balance (163) and is itself modulated by feeding in gastric neuroendocrine cells (164). Central TLQP-21 delivery does not affect feeding in mice but exerts an anorexigenic effect in Siberian hamsters (165) and predominantly stimulatory effects on the male hypothalamic-pituitary-gonadal axis (166). Chronic intracerebroventricular (icv) administration of TLQP-21 in mice increases energy expenditure and rectal temperature, an effect accompanied by increased serum epinephrine and decreased norepinephrine levels, but with no measurable changes in locomotor activity or free T_3 and free T_4 serum levels (163, 167). In addition, TLQP-21 treatment increased catabolic markers such as peroxisome proliferator-activated receptor δ, β-3 adrenergic receptor, and uncoupling protein 1 mRNA in white adipose tissue but had no effect on hypothalamic mRNA encoding metabolically active hypothalamic peptides (163, 168). Central delivery of TLQP-21 also prevented high-fat diet-induced obesity without any effect on food intake (163, 167). Jethwa *et al.* (165) confirmed a catabolic role for TLQP-21 in hamster, where central delivery exerts an anorectic effect, decreasing body weight and adipose fat mass. Surprisingly, the metabolic profiles of TLQP-21-treated mice and hamsters closely match the phenotype of VGF-KO mice (157, 158). An intriguing hypothesis to reconcile this apparent discrepancy has been proposed (168): one or more VGF-derived peptides have an anabolic role, increasing energy storage and opposing TLQP-21 effects, thus accounting for the phenotype of the germline VGF-KO mice. Data have been reported that support this model: VGF peptides TLQP-62, HHPD-41 (168), and NERP-2 (169) exert an orexigenic effect when injected icv. The VGF-derived amidated peptide, NERP-2, administered icv to rats and mice, rapidly stimulated food intake and increased body temperature, oxygen consumption, and locomotor activity via an orexin-dependent mechanism (170, 171). Treatment with anti-NERP-2 IgG decreased food intake, whereas NERP-2-induced effects were abrogated by administration of anti-orexin IgG or orexin receptor antagonists (171). Finally, NERP-2 did not induce food intake or locomotor activity in orexin-deficient mice (171).

C. Regulation of gastrointestinal function: VGF peptide TLQP-21

Anatomical, molecular, and pharmacological evidence has established that the VGF peptide TLQP-21 plays a prominent role in gastroenteric function (reviewed in Ref. 172). *In situ* hybridization studies showed that VGF mRNA is highly expressed in the myenteric plexus, with clear evidence of expression in the glandular portion of the stomach (173, 174). More directed characterization of the gastrointestinal tract demonstrated VGF immunoreactivity in nerve fibers of the peripheral system, in a subpopulation of neurons of the enteric plexus, and in enterochromaffin-like and somatostatin cells in rat stomach (164, 175, 176). Brancia *et al.* (164) also determined the abundance (162 ± 11 pmol/g) of TLQP peptides by ELISA and described a decreased (~50%) concentration of these peptides in rat stomach after prolonged fasting.

In vitro and *ex vivo* studies showed that TLQP-21 peptide dose-dependently induces contraction of gastric fundus strips but fails to induce contraction of stomach antrum or more distal gut portions such as jejunum and ileum (176). TLQP-21 also induced prostaglandin (PG)-E2 and PGF(2α) release from the mucosal layer, whereas fundus strip contraction was completely abolished by pretreatment with the cyclooxygenase (COX) inhibitors indomethacin or naproxen as well as PG antagonists (176).

Classical studies established that experimental ulcerative lesions, induced by sc administration of cysteamine, increased VGF mRNA in both sensory neurons of the nucleus tractus solitarius and in neurons of the dorsal motor nucleus of the vagus that directly project to the stomach (177, 178). In line with a potential involvement of TLQP-21 in the outflow pathway from central nervous system (CNS) to gastrointestinal tract, acute icv but not ip or iv administration of TLQP-21 inhibited gastric emptying in a time- and dose-dependent manner (176) and reduced ethanol-induced gastric lesions in rats (179). The TLQP-21 gastroprotective effect against ethanol injury

was accompanied by a significant increase in gastric PGE2 and COX-1 expression. The nitric oxide synthase inhibitor NG-nitro-L-arginine methyl ester (70 mg/kg, sc), the nonselective COX inhibitor indomethacin (10 mg/kg, orally), and capsaicin denervation removed TLQP-21 gastroprotection. Central TLQP-21 injection also inhibited gastric acid secretion (180), and both inhibition of gastric emptying (176) and gastric acid secretion (180) were prevented by indomethacin pretreatment. Overall, recent data therefore suggest that TLQP-21 centrally mediates gastroenteric functions by inducing the synthesis of PG. Additional studies should establish whether the biological activity of TLQP-21 that has been established *in vitro*, in isolated organ contraction assays, is paralleled by a similar functional role *in vivo*.

D. Regulation of prohormone convertase activity: 7B2 and proSAAS peptides

Regulation of PC catalytic activity is a mechanism shared by two granins, 7B2 and proSAAS, that has the potential to affect the relative levels of the protein substrates of PC2 and PC1/3, respectively, and the resultant peptides generated by precursor protein cleavage in the regulated secretory pathway (56, 181).

1. 7B2 protein and peptides

The granin 7B2 functions as a chaperone to regulate PC2 catalytic activity. First isolated in 1982 from pig anterior pituitary (182), the 7B2 protein sequence has been highly conserved evolutionarily, and the purified protein has an acidic pI of 4.9 and is processed into peptides (100). Interaction of pro-7B2 with pro-PC2 was subsequently demonstrated and possibly blocks premature activation of the PC2 zymogen in the secretory pathway (100, 183). Recent studies further demonstrate that 7B2 prevents PC2 unfolding and aggregation in the secretory vesicle, perhaps through a chaperone-like mechanism that may generalize to additional proteins in the regulated pathway because 7B2 is expressed more widely than PC2 (184). Processing of 7B2 at a site composed of five basic residues (R151–R155 of pro-7B2) releases a C-terminal peptide, 7B2CP, which like 7B2 functions as a potent inhibitor of PC2 catalytic activity *in vitro*, in the nanomolar range (98, 185, 186). Structure function analysis of this 31-amino acid peptide indicates that a C-terminal Lys-Lys pair is required for its initial binding to PC2 and its inhibitory activity. Once hydrolyzed at this site, the C-terminal inhibitory peptide is inactivated and dissociates from the catalytic site, and PC2 catalytic activity increases (187–189). However, it is unclear whether the 7B2CP peptide has similar PC2 inhibitory activity *in vivo*, in the secretory pathway, and if so, in which secretory compartment this peptide interferes with pro-PC2 conversion to PC2.

2. ProSAAS protein and peptides

Functional characterization of the neuroendocrine secreted protein proSAAS, identified in a screen of C-terminally extended peptide intermediates in the brains of Cpe^{fat}/Cpe^{fat} mice, revealed potent PC1/3 inhibitory activity *in vitro*, in the nanomolar range (190, 191). Structural comparison with 7B2 (99) and combinatorial peptide library screens (192) further identified a PC1-inhibitory hexapeptide, LLRVKR, in proSAAS that was located at the C-terminal end of the processing intermediate peptide designated PEN (99, 193, 194). Similar to 7B2, the precise functional roles of PEN ($proSAAS_{221–242}$) and PEN-LEN ($proSAAS_{221–260}$) *in vivo*, in the secretory pathways of neural, endocrine, and neuroendocrine cells, have until relatively recently remained unclear. Analysis of PEN-LEN expression in embryonic and adult brain, showing accumulation in embryonic d 15.5 whole-brain extracts at a developmental age when prodynorphin processing by PC1/3 does not occur, and undetectable levels in the adult brain when prodynorphin is processed by PC1/3 are consistent with *in vivo* functionality (195). Recent targeted ablation of the *ProSAAS* gene in mice by homologous recombination has demonstrated a role for proSAAS in fetal neuropeptide processing *in vivo*, and in adult body weight regulation and locomotion (195) (see also *Section V.B*). Adult proSAAS-KO mice, however, have normal hypothalamic peptide levels detected by quantitative peptidomics approaches and normal pituitary ACTH by gel filtration and RIA analysis, suggesting that PC1/3 activity in the adult is not affected by lack of proSAAS, nor were levels of PC1/3 or PC2 protein altered in whole brain or pituitary (195).

E. Regulation of hormone, neurotrophin, and/or neurotransmitter release: CgA peptide catestatin, SgII peptide secretoneurin, VGF C-terminal, and NERP peptides

1. CgA-derived peptide CST

The peptide CST was initially identified as the most potent endogenous antagonist to nicotinic cholinergic receptor that inhibits nicotine-evoked catecholamine secretion in an autocrine/paracrine fashion (76, 196). Subsequently, CST was found to act as a potent vasodilator *in vivo* in rat by stimulating the release of histamine (197). Such release of histamine by CST was also demonstrated *in vitro* from mast cells (198). CST also inhibits desensitization of catecholamine release induced by nicotine (199). The naturally occurring human variants of CST ($Gly^{364}Ser$, $Pro^{370}Leu$, and $Arg^{374}Gln$) displayed differential potencies toward inhibition of nicotinic cholinergic agonist-evoked catecholamine secretion from sympathochromaffin cells *in vitro* in the following rank order of

potency: $Pro^{370}Leu$ more than wild-type more than $Gly^{364}Ser$ more than $Arg^{374}Gln$ (196). *In vivo*, human carriers of the $Gly^{364}Ser$ allele had profound alterations in autonomic activity, in both the parasympathetic and sympathetic branches, and may be protected against the development of hypertension, especially in males (200). The impact that these CST-driven alterations in catecholamine secretion and autonomic activity have on cardiac physiology and BP is discussed in *Section IV.H*.

2. SgII-derived peptide SN

In vitro studies demonstrated that SN induces dopamine release from rat striatal slices in a dose- and calcium-dependent manner (201). These results were corroborated *in vivo* by microdialysis experiments (202). You *et al.* (203) extended these studies to the substantia nigra and neostriatum where treatment with SN increased the release of dynorphin B and classical transmitters like dopamine, glutamate, and γ-aminobutyric acid. In the endocrine system, a regulatory action of SN on the pituitary was observed; SN stimulated LH release and synthesis in goldfish gonadotrophs (204) and the mammalian LβT2 cell line (205). In contrast to GnRH, treatment with SN in the low nanomolar range specifically induced LHβ but not FSH release from LβT2 cells. Additional effects of SN on immune, endothelial smooth, and muscle cells are discussed below (see *Section IV.G*).

3. VGF C-terminal and NERP peptides

The VGF-derived NERP-1 and NERP-2 were initially identified in a screen for biologically active, C-terminally amidated peptides from human medullary thyroid carcinoma TT cells (169). NERP are highly abundant in rat hypothalamus and colocalize with vasopressin in storage granules, and consistent with a role in the regulation of water balance, VGF mRNA levels in both paraventricular nucleus of the hypothalamus (PVN) and supraoptic nucleus are regulated by water deprivation, and NERP-1 suppresses angiotensin II-induced vasopressin secretion from PVN and supraoptic nucleus in hypothalamic explants (169). As noted in *Section IV.B*, Nakazato and colleagues (171) have shown that NERP-2 administered into rats or mice increases food intake via an orexin-dependent mechanism, suggesting that this VGF-derived peptide also functions by selectively stimulating the release of the neuropeptide orexin in specific hypothalamic circuits.

The C-terminal VGF-derived peptides TLQP-62 and AQEE-30 have been demonstrated to increase synaptic activity in cultured hippocampal neurons (206), whereas TLQP-62 stimulates electrical potentiation in hippocampal slices (207) and dorsal horn neuron excitability in spinal cord slices (208). In hippocampal slices, TLQP-62-induced electrical potentiation was selectively blocked by the brain-derived neurotrophic factor (BDNF) scavenger TrkB-Fc, Trk tyrosine kinase inhibitor K252a, and tissue plasminogen activator STOP, which inhibits tissue plasminogen activator, an enzyme involved in pro-BDNF cleavage to BDNF (207). These data suggest that TLQP-62 may function in part by selectively stimulating release of BDNF in the hippocampus, and perhaps in other regions of the CNS. Consistent with this model, AQEE-30 and the shorter C-terminal peptide LQEQ-19 activate microglia and stimulate phosphorylation of MAPK p38 (209), critical steps in nociceptive signaling that could induce BDNF release from microglia after injury, which in turn has been shown to mediate changes in dorsal horn neuronal excitability (210).

F. Regulation of neural pathways that control pain, emotion, and sexual behavior: VGF- and CgA-derived peptides

1. Pain

VGF is abundantly expressed in neurons of both sympathetic and spinal sensory ganglia (175). Positive immunostaining for VGF is observed in the spinal cord, particularly in the superficial dorsal horn and in the region surrounding the central canal and in many neuronal cell bodies of the spinal ganglia (175). Recently, increased VGF mRNA and protein levels have been observed in dorsal root ganglia and spinal cord after sciatic nerve transection or in other neuropathic pain models (208, 209, 211–213). In dorsal root ganglia sensory neurons or in dorsal horn, VGF colocalizes with substance P, calcitonin gene-related peptide (CGRP), TrkA, and P2X3 (209, 214). Intraplantar or intrathecal delivery of C-terminal VGF-derived peptides (TLQP-21, TLQP-62, AQEE-30, or LQEQ-19) consistently induces hyperalgesia or hypersensitivity in different models of pain (208, 209, 214). One of these studies showed that icv delivery of TLQP-21 exerts an analgesic effect in the forepaw-injected formalin test (214). Riedl *et al.* (209) further established that thermal hyperalgesia mediated by AQEE-30 and LQEQ-19 was dependent upon the activation of microglial p38 MAPK.

Intraperitoneal delivery of the CgA_{4-16} peptide does not directly modulate pain but rather increases the number of abdominal constrictions induced by ip acetic acid administration (215). The pronociceptive effect induced by CgA_{4-16} was blocked by pretreatment with L-type calcium channel antagonist diltiazem or the COX-2 inhibitor indomethacin. In addition, CgA_{4-16} potentiated CGRP- and capsaicin-induced abdominal writhing but not that evoked by substance P. Similar investigation of CgA_{47-66} inflammatory activity revealed dose-dependent effects; below 0.5 mg/kg ip, administration pro-

duced antinociceptive effects, whereas at 2 mg/kg, it produced a pronociceptive effect on acetic acid-induced abdominal pain in rats (216). Pronociceptive effects of CgA_{47-66} were also blocked by diltiazem and indomethacin, and writhing evoked by CGRP or capsaicin was abolished by CgA_{47-66} (216).

2. Emotional behavior and psychiatric disease

In humans, schizophrenia has been associated with lower levels or no change in CgA, CgB, and SgII in cerebrospinal fluid (CSF) (217–221). VGF mRNA levels are also reduced in peripheral leukocytes of drug-free depressed patients (222) and in discrete areas of postmortem brain obtained from patients with bipolar disorder (223) but are unchanged in postmortem brain from patients who suffered from major depression or schizophrenia (223). In contrast, increased CSF content of VGF_{23-62} was reported in first-onset drug-naive schizophrenic patients (224, 225).

Of the granin proteins, VGF has been perhaps the most extensively investigated for its role in emotional behavior and psychiatric disease. Preclinical studies in rodents demonstrate that VGF levels are up-regulated by antidepressant drugs and voluntary exercise and are reduced in animal models of depression and antidepressant efficacy, including in learned helplessness and the forced swim test (222, 226, 227). VGF-derived peptides TLQP-62 and AQEE-30, administered icv or into the hippocampus in rodents, exert an antidepressant-like effect in the forced swim test, tail suspension test, and novelty-induced hypophagia test (226, 227), whereas neither peptide affects anxiety- or novelty-induced locomotor activity (226, 227). Although the mechanisms of these antidepressant actions remain to be fully elucidated, C-terminal VGF peptides such as TLQP-62 and AQEE-30 have been shown to enhance hippocampal synaptic plasticity as well as neurogenesis in the dentate gyrus (206, 207, 227). Consistent with the antidepressant efficacy of VGF peptides, VGF-deficient mice have increased immobility in the forced swim and tail suspension tests while showing impairments in both contextual fear-conditioned learning and spatial learning in the Morris water maze (207, 226).

Currently, there are few data to support a role for other granin proteins in emotional behavior. The icv administration of SgII-derived peptides GE-19, GAIPIRRH, and SN exerted no or minimal effect on anxiety- and novelty-induced activity in rats and mice (228) despite inducing release of dopamine from rat striatal neurons (201, 202).

3. Sexual behavior

Injection of C-terminal VGF-derived peptides AQEE-30 and LQEQ-19 into the PVN stimulates nitric oxide production in the PVN and facilitates penile erection in rats (229, 230). VGF-induced penile erection is partially inhibited by pretreatment with the nitric oxide-synthase inhibitor L-NG-nitro-L-arginine methyl ester, the oxytocin receptor antagonist *d*-[CH (2)](5)Tyr(Me)-Orn(8)-vasotocin, morphine, and muscimol (229, 230). VGF-derived peptides therefore modulate male erectile behavior and gonadotropin responses of the male hypothalamic-pituitary-gonadal axis (TLQP-21) (166), consistent with infertility observed in male VGF-KO mice (158).

G. Regulation of the immune system: CgA, SgII, and their peptides

Relevant to the immune and cardiovascular systems, a potent chemotactic activity of SN toward monocytes, eosinophils, and endothelial cells was established (87). Like SN, CST also stimulated chemotaxis of human peripheral blood monocytes, exhibiting its maximal effect at 1 nM, which was comparable to the established chemoattractant formylated peptide Met-Leu-Phe (fMLP) (231). This finding indicated that CST could be considered an inflammatory cytokine. Recent studies further demonstrate that both CST and the antimicrobial peptide chromofungin, comprising the third amphipathic helix of the CgA-derived peptide VST-1, induce calcium entry into human neutrophils (232). These studies establish a pathway by which endocrine and immune systems could communicate. In addition, CST was found to induce chemotaxis in human umbilical vein endothelial cells with a maximal effect at 1 nM, comparable to angiogenic vascular endothelial growth factor (VEGF) or SN (233). Moreover, the local presence of SN within the rat CNS influenced the topographical distribution of inflammatory cell infiltrates in acute T-cell-mediated encephalomyelitis (234). Clustering of macrophages, but not of T lymphocytes, was observed at sites of SN immunoreactivity in all stages of experimental autoimmune encephalomyelitis, suggesting a proinflammatory role for SN *in vivo*. In contrast to SN, which activates inflammatory cell migration and extravasation, the CgA-derived peptide VST-1 prevents vascular leakage and VEGF-induced endothelial cell migration (235–237). In addition to regulating immune cells, a number of granin-derived peptides, including chromofungin (CgA_{47-66}) and CST, directly inhibit growth of fungi, yeast, and bacteria. Contributions of granins and granin-derived peptides to inflammatory conditions and innate immunity have recently been reviewed (238, 239).

H. Regulation of blood pressure, angiogenesis, and the cardiovascular system: CgA, SgII, and their peptides

1. Hypertension

Because excess sympathetic activity is implicated as a cause of hypertension, and basal plasma CgA concentra-

tion is correlated with sympathetic tone (240, 241), one would expect that mechanisms involving CgA and the CgA peptide CST might be altered in hypertension or in individuals at risk for the development of hypertension. Compared with age-matched normotensive controls, patients with essential hypertension have increased plasma CgA (242). It has also been shown that plasma CST is diminished not only in established cases of essential (hereditary) hypertension but also in the still-normotensive offspring of patients with hypertension (243), indicating that an early deficiency in CST might play a pathogenic role in the subsequent development of hypertension. Subjects with such a family history demonstrate increased epinephrine secretion in addition to diminished CST (243), indicating that CST exerts an inhibitory effect on chromaffin cells *in vivo*. Taken together, these findings suggest a complex relationship between BP and the expression of CgA and its peptides, CST and PST (see also *Section VI.B*). Recent studies indicate an inverse relationship between circulating levels of CgA and CST (244). Consistent with findings in humans, targeted ablation in mice of the *Chga* gene resulted in high BP that was rescued by treatment with CST (137) (see *Section V.B*).

Later studies revealed a more complicated biphasic (or U-shaped) dose-response curve for *Chga* gene copy number and basal BP, and parallel changes in adrenal catecholamine storage and release (245). High or low CgA expression in mice and humans was associated with maximal BP and maximal BP responses to cold stress, respectively (245). Thus, these studies indicate that CgA plays a critical role in the regulation of BP, but the relationship between CgA levels and BP is not strictly linear and is also dependent on the extent of CgA processing into its bioactive peptides. Moreover, a number of variants of the human CgA peptide CST have been characterized (see *Section V.A*), that differentially affect catecholamine release and hypertension risk (200). In addition to inhibiting catecholamine secretion with different potencies, these variant CST peptides are processed from CgA with different efficiencies (246). Lastly, reduced conversion of CgA to CST has been measured in hypertensive patients, and independent genetic loci have been identified in twin studies that influence BP through diminished CgA processing to CST (244). One of these encodes a subunit of the vesicular H(+)-translocating ATPase, a protein that regulates secretory vesicle acidification, and CgA sorting, secretion, and processing (244). A minor SNP variant of this ATPase is associated with increased systolic BP (244).

2. Angiogenesis

The CgA-derived peptides CST and VST-1 and the SgII-derived peptide SN have been linked to vasculogenesis and remodeling, also a potential contributor to hypertension. CST induces migration and proliferation of endothelial cells and stimulates chemotaxis in vascular smooth muscle cells or endothelial progenitor cells *in vitro*. Pronounced angiogenic and vasculogenic activities of SN and CST, comparable to that of VEGF, were identified *in vivo* in the mouse cornea system and *in vitro* in a Matrigel tube formation assay (233, 247–249). CST also exhibits vasorelaxant and antihypertensive effects through its interaction with histamine receptors (197). Delivery of SN via an expression vector or im injection of CST resulted in greatly improved clinical outcomes in mice with hind-limb ischemia induced by surgical ligation of the femoral artery (233, 250). SN also stimulated proliferation and exerted antiapoptotic effects on endothelial cells via activation of the ERK and Akt signaling pathways (233, 247). Furthermore, in an *in vivo* stroke model, iv administration of SN after occlusion of the right middle cerebral artery of rats led to reduced infarct volume and improved motor performance (251). In contrast to CST and SN, VST-1 inhibits VEGF-induced endothelial cell proliferation and migration and the formation of capillary-like structures (237). However, similar to CST, VST-1 has vasorelaxant properties (252).

3. Cardiac contractility

Another pathophysiological mechanism that could impact BP is the direct regulation of cardiac muscle contractility. In addition to its synthesis and secretion from the adrenal medulla, CgA and its peptides are expressed in the human heart (253). Importantly, in addition to its vasorelaxant properties, VST-1 has negative inotropic and lusitropic effects on the heart, which have been proposed to stabilize the cardiovascular system under conditions of stress, including sympathetic β-adrenergic overstimulation and cardiac injury. Interestingly, CST also has direct myocardial and coronary effects, reducing contractility (254). In the isolated working frog heart, CST acts as an important cardioinhibitor, reducing both stroke volume and stroke work (255). In fish heart, CST modulates myocardial function (negative inotropism), both under basal and increased preload conditions, and is able to counteract β-adrenergic-mediated positive inotropism (256). Due to space constraints, these important cardiovascular effects, investigated in a variety of vertebrate species, cannot be detailed here but have been beautifully described in several recent reviews (257, 258).

V. Genetic Insights into Granin Function

Studies of SNP in granin genes and their disease implications (*Section V.A*), transgenic and KO mouse models

(*Section V.B*), and the potential for simpler genetic model organisms to provide substantive new insights into granin protein function (*Section V.C*), are reviewed below.

A. *CHGA* and *CHGB* genetic variants (SNP)

1. CHGA and CHGB coding sequence variants

Using systematic polymorphism discovery at the human *CHGA* (*CgA*) locus in 180 subjects from diverse biogeographic ancestries, three human variants of CST were identified that showed differential potencies toward inhibition of catecholamine secretion ($Pro^{370}Leu$ > wild-type CST > $Gly^{364}Ser$ > $Arg^{374}Gln$) (196, 259). In the Langendorff perfused rat heart preparation, the $Gly^{364}Ser$ variant was found to abolish isoproterenol-induced positive inotropism and lusitropism (254). In humans, Gly/Ser heterozygotes displayed increased baroreceptor slope during upward deflections (by ~47%) and downward deflections (by ~44%), increased cardiac parasympathetic index (by ~2.4-fold), and decreased cardiac sympathetic index (by ~26%) (200). The $Gly^{364}Ser$ variant was associated with lower diastolic BP in two independent/confirmatory groups of patients with hypertension; genotype groups differed by approximately 5–6 mm Hg, and the polymorphism accounted for approximately 1.8% of population diastolic BP variance. The CST $Gly^{364}Ser$ variant therefore causes profound changes in human autonomic activity, both parasympathetic and sympathetic, and seems to reduce risk of developing hypertension, especially in men.

In the Chinese population, the $Arg^{533}Gln$ variant in the *CHGB* (*CgB*) gene was found to be associated with schizophrenia (260). However, in the Japanese population, both the nonsynonymous ($Arg^{353}Gly$) and synonymous ($Glu^{368}Glu$) *CHGB* variants were reported to be associated with schizophrenia (261).

2. CHGA and CHGB variants in the promoter region

Five tightly linked common promoter variants (at positions C-1014T, G-988T, G-462A, C-415T, and A-89C) were investigated in a study of 112 phenotyped twin pairs, and haplotypes were inferred with the HAP algorithm (262). The three most common haplotypes, TTGTC (Hap-A, 56.9%), CGATA (Hap-B, 23.0%), and TTGCC (Hap-C, 16.5%), accounted for more than 95% of all haplotypes. Diploid haplotypic variation in the region markedly affected both the change in diastolic BP and the final diastolic BP during cold stress (263). Homozygosity for Hap-B (CGATA/CGATA) seemed to blunt both change in diastolic BP and final diastolic BP, whereas Hap-A/Hap-B heterozygosity (TTGTC/CGATA) was associated with the greatest values of both change in diastolic BP and final diastolic BP. The more extreme trait values for heterozygotes (compared with either homozygote class) suggested the phenomenon of molecular heterosis (264). There are two common SNP in the proximal promoter of human *CHGB*: A-296C and A-261T. Like *CHGA*, *CHGB* promoter variant (A-261T) was found to be associated with hypertension especially in men (265). In addition, *CHGB* promoter polymorphisms A-296C/A-261T interact nonadditively to influence systolic BP and diastolic BP (265).

3. CHGA variant in 3′-UTR region

A common (~27% frequency) genetic variant in the *CHGA* 3′-UTR (C+87T) is found to be strongly associated with human essential hypertension, accounting for up to approximately 12/9 mm Hg of BP variation within the population (266). This association is substantially more impressive in men than in women, effectively confining the effect of C+87T to men. The 3′-UTR variant also predicts environmental stress-induced increments in BP, suggesting a mechanism for early effects of the gene on a pathogenic series of events eventuating in sustained BP elevation (266).

B. Mouse models (transgenic and knockout)

1. CgA, CgB, and SgII

Chromogranins/secretogranins constitute the major soluble proteins in the vesicular matrix of catecholamine storage vesicles or CG and LDCV (9). These proteins are costored and coreleased with neurotransmitters. To gain better insight into the functions of chromogranins and secretogranins, including intracellular roles regulating catecholamine storage in the CG/LDCV and in granule biogenesis and extracellular functions mediated by a number of bioactive peptides, CgA (137) and CgB (139) KO mice have been generated.

Patch amperometry, which monitors vesicle size (capacitance) and the release of catecholamines from the same vesicle (amperometry), on primary chromaffin cells from CgA-KO mice revealed an approximately 40% drop in vesicular concentration of catecholamines (267). These findings indicate that CG from chromaffin cells lacking CgA possesses dramatically weaker capacity to accumulate catecholamines. Using amperometry as the method of quantification, it was shown that CG from chromaffin cells of CgA-KO mice release 40% less catecholamine than those from wild-type mice after a depolarizing stimulus with comparable spike pattern. Catecholamine release per quantum was reduced (by 34%) in CgA-KO mice. The kinetic analysis of secretory spikes showed that exocytosis occurred faster in CgA-KO cells, affecting mainly the last part of the spikes. These findings indicate that the matrix of LDCV without CgA is less capable of concentrating and

retaining catecholamines, causing exocytosis to occur faster, eventuating in higher plasma (137) or urinary (138) catecholamines. Similarly, CG from chromaffin cells of CgB-KO mice also show decreased catecholamine release (by 33%) coinciding with the amount released per quantum (268). These findings indicate that chromogranins/secretogranins are required to concentrate catecholamines inside CG.

a. Chromogranins/secretogranins as regulators of CG biogenesis and sorting of secretory proteins. In 2001, Loh's group provided evidence that CgA plays a crucial role in biogenesis of secretory granules (see *Section III.C*). Impairment of CgA expression by antisense RNA depleted secretory granules, inhibited regulated secretion of a prohormone, and reduced secretory granule protein in cells (30). Consistent with *in vitro* studies, targeted ablation of the *Chga* gene resulted in the loss of CG (137). CgA antisense transgenic mice also showed a significant reduction in CG in adrenal chromaffin cells caused by down-regulation of CgA (136). The number of CG formed was directly proportional to the amount of CgA present in adrenal medulla. These findings confirm and extend earlier reports and clearly demonstrate a critical role for CgA in the biogenesis of CG (see also *Section III.C*).

b. Chromogranins/secretogranins as regulators of cardiac homeostasis and hypertension. Targeted ablation of the *Chga* gene in KO mice resulted in high systolic BP, elevated plasma catecholamines (137), increased reactive oxygen species, and decreased nitric oxide (269). Rescue of elevated BP to normalcy was achieved by either exogenous replacement or humanization of CgA-KO mice (137, 270). Loss of the physiological brake CST in CgA-KO mice coupled with dysregulation of transmitter storage and release may act in concert to alter autonomic control of the circulation *in vivo*, eventuating in hypertension (137). So although elevated plasma CgA levels are associated with hypertension, germline ablation experiments in mice suggest that the predominant physiological role of CgA is to reduce BP, most likely via CST. Consistent with this model, CST replacement rescued CgA-KO mice from their high resting BP and normalized plasma catecholamine levels. CST replacement also selectively diminished stress-induced increments in BP and heart rate in CgA-KO mice, implicating CST as an antihypertensive peptide even in stressful conditions.

A related result was found by experimental disruption of CgB expression. Targeted ablation of the *Chgb* locus in CgB-KO mice elevated systolic and diastolic BP by 20 and 18 mm Hg, respectively (265). This is consistent with the inverse relationship between circulating CgB levels and catecholamine secretion (see *Section VI.B.2*).

c. Chromogranins/secretogranins as regulators of insulin sensitivity. PST acts as an antiinsulin peptide (see also *Section IV.A*), inhibiting most of the actions of insulin, so one would expect CgA-KO mice to be more sensitive to insulin owing to loss of this CgA-derived peptide. Thus, CgA-KO mice are euglycemic despite low plasma insulin and high plasma catecholamine levels, because of increased liver insulin sensitivity. Moreover, PST replacement in these mice increased blood glucose by stimulating phosphoenolpyruvate carboxykinase and glucose-6-phosphatase mRNA abundance, reducing the suppressive effect of insulin on hepatic gluconeogenesis (156). Although both CgA and CgB are conserved at the N- and C-terminal ends, CgB-KO mice show marked resistance to insulin in contrast to increased insulin sensitivity in CgA-KO mice. Identification of an insulin-sensitive CgB peptide would explain the findings in CgB-KO mice.

d. Chromogranins/secretogranins as regulators of type 1 diabetes. Although many laboratories have identified $CD4^+$ T cell clones in nonobese diabetic (NOD) mice that are reactive to pancreatic antigens *in vitro* and that also cause or accelerate diabetes in various types of *in vivo* experiments, the antigenic targets of other highly pathogenic $CD4^+$ T cell clones have remained elusive. The most extensively studied of these are the BDC clones (BDC is a clonal designation for islet-specific T-cell clones) (271), which were isolated from the spleens and lymph nodes of diabetic NOD mice and include BDC-2.5. Initially, CgA was identified as the source of the antigen for BDC-2.5 and two other clones, because it was missing from CgA-KO pancreatic islet cells. Further in-depth studies led to the identification of the CgA peptide WE14 as the autoantigen for type 1 diabetes (77). It is thus clear that CgA-KO mice have provided significant insights into the function of PST and WE14 as regulators of type 1 and 2 diabetes.

2. 7B2 knockout mouse model

Using biochemical and cell biological approaches, 7B2 was found to be a critical chaperone for PC2 and regulator of its catalytic activity (100). To test protein function *in vivo*, exon 3 was disrupted by random integration of a transposon into the *7B2* gene in mice (101); analysis of this line supported a key role for 7B2 in PC2 activation, although also revealing additional important contributions to the regulation of pituitary hormone secretion. Homozygous 7B2-KO mice lack PC2 activity and, in addition, die before 9 wk of age from a severe Cushing's syndrome that results from pituitary ACTH hypersecretion (101), a consequence of interrupted PC2 processing. The 7B2-KO

mice are hypoglycemic, hyperproinsulinemic, and hypoglucagonemic, with a number of metabolic and endocrine abnormalities (101, 272) that are largely reversed by adrenalectomy (102) or adenoviral-mediated expression of 7B2 in the pituitary (272). 7B2- and PC2-KO mice share similarities in glucose homeostasis and have depressed enkephalin and glucagon levels and increased pituitary ACTH, but the phenotypes differ in that 7B2-null mice develop severe pituitary Cushing's disease, adrenal cortical hyperplasia, and increased circulating ACTH (101), the latter likely a result of decreased dopamine levels and inhibitory control in the intermediate lobe of the pituitary (102). Loss of 7B2 therefore has an overlapping yet more substantial effect on the function of the regulated secretory pathway in the pituitary than does PC2 ablation, suggesting potentially novel roles for 7B2 beyond PC2 catalytic regulation.

3. VGF knockout mouse model

VGF was identified on the basis of its rapid, robust transcriptional regulation by neurotrophic growth factors (273–275) and is secreted from neuronal, neuroendocrine, and endocrine cells (109, 276, 277). Unlike other granins, initial elucidation of VGF function relied largely on the development of a KO mouse model (158). Although indistinguishable from littermates at birth, adult homozygous germline VGF-KO mice are lean and hypermetabolic, 50–70% the size of wild-type mice, and resistant to diet- or lesion-induced and specific forms of genetically induced obesity and diabetes (157, 158, 160). Food intake is similar in absolute terms although increased per gram of body weight in VGF-KO mice, which is consistent with endocrine changes such as increased corticosterone levels and increased insulin sensitivity (158, 160). In VGF-KO mice, hypothalamic levels of NPY and AGRP mRNA are elevated by 600 and 800%, respectively, although POMC mRNA is reduced by 75% in comparison with controls, compatible with a fasting state (158). Importantly, VGF-KO mice are fully resistant to obesity induced by diet, gold thioglucose treatment (a hypothalamic lesion-induced form of obesity), and ectopic overexpression of agouti ($A^{y/a}$ mice) or targeted deletion of the melanocortin 4 receptor (melanocortin 4 receptor KO mice) (157, 158, 160). VGF-KO mice are partially resistant to obesity induced by leptin deficiency (*ob*/*ob* mice) although remaining vulnerable to obesity induced by monosodium glutamate administration to neonatal mice (which damages the hypothalamus and sympathetic nervous system) (157, 160). Overall, these data suggest that VGF has a functional role in projections of the sympathetic nervous system that innervate metabolic tissues (109, 157). Recent studies further demonstrate a *Vgf* gene-dosage-dependent effect on lipid storage and adipocyte cell size and increased mitochondrial number, cristae number, and uncoupling protein expression in VGF-KO brown adipose tissue, providing a possible mechanism for their higher metabolic rate (159). As noted in *Section IV.B*, the phenotype of germline VGF-KO mice is not totally consistent with the effects of certain icv-administered VGF peptides on energy balance. Future conditional ablation of the *Vgf* gene in the adult nervous and endocrine systems may help to clarify distinct roles for this granin during development and in the adult.

Perhaps more in line with the cloning of VGF as a neurotrophin-inducible gene product, and the role that BDNF plays in the regulation of synaptic plasticity and behavior, heterozygous and homozygous VGF-KO mice have deficits in memory and emotional behavior (207, 226). In this case, the antidepressant/anxiolytic efficacy of VGF-derived C-terminal peptides AQEE-30 and TLQP-62 (223, 226, 227) is consistent with the prodepressive phenotype of VGF heterozygous KO mice (226) and their resistance to the antidepressant effect of voluntary exercise in the forced swim and tail suspension tests (226). VGF is also required for some of the behavioral effects and signal transduction pathway activation that is stimulated by LiCl treatment (223). Potentially relevant to antidepressant efficacy, TLQP-62 regulates hippocampal progenitor proliferation (227) and stimulates electrical potentiation in hippocampal slices via a BDNF-dependent mechanism (207), although both TLQP-62 and AQEE-30 increase electrical excitability of dissociated hippocampal neurons (206). Future experimentation to better delineate VGF function in emotional behavior, memory, and energy metabolism will likely blend *in vivo* and *in vitro* peptide administration studies with the analysis of germline and conditional KO mice.

4. ProSAAS knockout mouse models

Similar to 7B2, proSAAS was initially functionally characterized as a potent PC1/3 inhibitor *in vitro*, using biochemical and cell-based assays (see *Section IV.D.2*). However, the physiological impact of proSAAS *in vivo* was clarified only recently through the analysis of transgenic (278) and KO (279) mouse models. Approximately 2-fold overexpression of proSAAS mRNA, driven by the β-actin promoter in transgenic mice, led to a delayed increase in body weight and fat mass and slightly elevated fasting blood glucose levels in adult 10- to 12-wk-old mice, which did not appear to be due to hyperphagia (278). Because the phenotypes of PC1-KO mice (280) and proSAAS transgenic mice are quite distinct and pituitary peptide processing in proSAAS transgenic mice appeared normal, overexpression of proSAAS in this transgenic line

was felt to be insufficient to substantially impair PC1 activity in the pituitary. As a result, it is likely that proSAAS and/or peptides cleaved from this precursor have biological activities that account for the moderate, late-onset obesity in transgenic mice and that these are independent of an effect on PC1 activity (278). To further assess proSAAS function *in vivo*, a germline KO line was subsequently generated and analyzed (279). Fetal proSAAS-KO mice exhibit complete, adult-like processing of prodynorphin in the prenatal brain, rather than incomplete processing, the result of inhibitory proSAAS intermediates affecting PC1 activity, which is seen in brains of wild-type fetal mice. In adult mice lacking proSAAS, PC1/3 activity does not appear to be affected, but these mice do exhibit decreased locomotion and a male-specific 10–15% decrease in body weight, again arguing that proSAAS and/or proSAAS-derived peptides have bioactivities that are independent of this protein's inhibition of PC1/3 catalytic activity (279).

C. Nonmammalian vertebrate and invertebrate model organisms

Analysis of granins in lower vertebrates has proven incredibly useful in defining evolutionary conservation of granin structure and function, as discussed in *Section II* and comprehensively reviewed (45). Simple genetic models such as zebrafish and *C. elegans* offer the added advantage of more tractable genetic manipulation, coupled to the analysis of functional outcomes. Biochemical analysis of *Xenopus* and zebrafish (*D. rerio*) proSAAS proteins indicates that these nonmammalian molecules inhibit mouse PC1/3 with nanomolar inhibition constants and exhibit neural and endocrine distributions, suggesting functional conservation (51). Similar studies have demonstrated conservation of 7B2 function in *C. elegans* (281, 282) and *Drosophila* (283), in addition to higher vertebrate organisms (100), all of which express PC2. Compared with 7B2 and proSAAS, it has been difficult to biochemically test phylogenetic conservation of CgA, CgB, and SgII function; however, expression and distribution of chromogranin transcripts, proteins, and chromogranin-derived peptides have been examined. Using one- and two-dimensional electrophoresis coupled with immunoblotting (284), or immunohistochemistry (285–287), investigators were able to demonstrate CgA-like, SgII-like, and/or CgB-like proteins in mammals, birds, amphibians, fish, and arthropods and CgA mRNA in zebrafish (288). Future studies in some of these simpler genetic model organisms, including zebrafish and *C. elegans*, which are amenable to RNA knockdown experimental strategies, should provide complementary functional data to that obtained using KO mouse models.

VI. Granins as Disease Biomarkers

Biological markers (*i.e.*, biomarkers) are characteristics that can be objectively measured and evaluated as an indicator or surrogate endpoint of a biological process, pathophysiological process, or a response to pharmacotherapeutic intervention (289). The identification of clinically useful biomarkers can therefore have a profound impact on disease diagnosis and treatment, our understanding of disease pathogenesis, and the development of new drugs. The widespread utility of granin-derived peptides in serum and CSF as biomarkers of specific diseases, including hypertension, neurodegenerative disease [*e.g.*, amyotrophic lateral sclerosis (ALS) and Alzheimer's disease], neuropsychiatric disease (*e.g.*, depression, schizophrenia, and bipolar disease), and cancer, and more recently to establish therapeutic efficacy during drug development, is reviewed below (see also Refs. 48, 52, and 290–293). The diversity of granin biomarkers is evident in Table 4, where therapeutic utility in endocrine and neuroendocrine tumor diagnosis and prognosis are summarized, and in Fig. 7 and accompanying Table 5, where specific contributions of granin biomarkers to neuropsychiatric, neurological, and cardiovascular disease are explored.

A. Endocrine and neuroendocrine tumors

Chromogranin and secretogranin proteins, and their proteolytically processed peptides, are often used to identify specific tumors with a secretory phenotype (*e.g.*, carcinoid tumors) (294, 295), allowing one to assess a tumor's degree of malignancy and metastatic potential, and to distinguish primary from metastatic lesions. For example, the SgII-derived peptide EM66 selectively discriminates benign from malignant pheochromocytoma (296, 297), although the SgII-derived peptide SN is a specific marker for endocrine pancreatic tumors (298). NESP55 staining has utility in predicting the primary lesion site of metastatic disease, distinguishing pancreatic tumors and pheochromocytomas (299) from other neuroendocrine carcinoid tumors that are derived from gastrointestinal tract or lung (300, 301).

Sometimes the loss of negative regulators or tumor suppressors (302–304) can reactivate an endo-exosecretory phenotype leading to an increase in secreted peptides. Increased circulating peptide levels parallel tumor mass and metastasis development, a negative prognostic indicator in prostate carcinoma (305) or gastrointestinal tumors (306). Also, for gastroenteropancreatic neuroendocrine tumors expressing somatostatin receptors, decreased plasma CgA is a reliable marker of patients who are most likely to respond to treatment (306). In other cases, the secreted granin peptides are diagnostic

TABLE 4. Studies relating to granins as disease biomarkers for endocrine and neuroendocrine tumors

	CgA	CgB	SgII	SgIII	7B2	NESP-55	VGF	ProSAAS
Breast cancer	307, 308, 389	307, 308, 389	N/A	N/A	N/A	N/A	302, 416	N/A
Gastroenteric tumors	290, 295, 306, 390, 391	290, 295, 306, 403, 404	407–409, 376	376	N/A	300, 301	417	N/A
Insulinoma	295, 391, 392	391, 392	298, 376	376	412	299	417, 418	N/A
Liver neoplasia	393	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Lung tumors	394	388	410	303	413	300, 301	419	N/A
Medullary thyroid carcinoma	295, 395	404	N/A	376	N/A	N/A	417	N/A
Medulloblastoma	N/A	N/A	N/A	N/A	304	N/A	N/A	N/A
MEN and von Hippel-Lindau syndrome	396, 397	405	N/A	N/A	414	N/A	N/A	N/A
Neuroblastoma and ganglioneuroma	398, 399	N/A	398	N/A	N/A	294, 299	420	N/A
Parathyroid adenoma	295	N/A	N/A	N/A	N/A	N/A	417	N/A
Pheochromocytoma	295, 311, 361	311, 406	311, 296, 297	N/A	311, 412	294, 299, 300, 311	311, 417	311, 421
Pituitary adenoma/ carcinoma	290, 400, 401	N/A	N/A	N/A	415	N/A	417	N/A
Prostate cancer	305, 402	402	402, 411	N/A	N/A	N/A	N/A	N/A

MEN, Multiple endocrine neoplasia; N/A, no published study available.

biomarkers of differentiated neuroendocrine tumors, for example in breast carcinomas (307, 308) or pheochromocytomas (309), where they are indicative of a better prognosis. Table 4 summarizes the application of granin biomarkers to endocrine and neuroendocrine tumor diagnosis, which has been the subject of a number of excellent recent reviews (290–292, 294, 295, 310, 311).

B. Cardiovascular disease and hypertension

1. CgA biomarkers

The sympathoadrenal system exerts minute-to-minute control over cardiac output and vascular tone. Basal plasma CgA concentration is correlated with sympathetic tone (240, 241), and studies in twins indicate that the basal level is highly heritable (242). As compared with age-matched normotensive controls, patients with essential hypertension have increased plasma CgA and an increased release of stored CgA in response to adrenal medullary stimulation by insulin-evoked hypoglycemia (242). Consistent with these findings in humans, expression of the *Chga* gene was observed to be significantly higher in adrenal glands of rat and mouse models of genetic hypertension (312–314), supporting the phenotypic association between elevated CgA and essential hypertension. The dysglycemic CgA fragment PST is also elevated in patients with essential hypertension with or without obesity (315, 316); its actions may therefore contribute to the insulin resistance that often accompanies this condition. In contrast, CST is decreased in patients with essential hypertension and even in normotensive subjects with a family history of hypertension (243). Low CST levels also predict augmented adrenergic response to stressors, suggesting that reduction in CST increases risk of hypertension (243).

Several recent clinical studies have extended the potential use of CgA as a disease biomarker to a broad range of cardiovascular diseases ranging from myocardial infarction to heart failure. Specifically, plasma CgA showed a robust association with mortality risk after myocardial infarction or acute coronary syndrome (317–319) as well as heart failure (318, 320–322). Similar data were also obtained in a mouse model of chronic heart failure (323), and in a recent preclinical study using isolated perfused rat hearts, CST was found to reduce myocardial infarct size and postischemic rise of diastolic left ventricular pressure and to significantly improve postischemic recovery. Furthermore, in isolated cardiomyocytes, CST increased the cell viability rate by about 65% after simulated ischemia/reperfusion (324).

These observations suggest a clinical consensus for the prognostic value of CgA in heart disease (320, 325). It should be highlighted, however, that contrasting findings have been reported (323, 326). However, from a biomarker and physiological viewpoint, the association between CgA and cardiovascular disease likely reflects the contributions of sympathetic function to the disease process.

2. CgB biomarkers

Expression of CgB may mark the action of still poorly characterized trans-quantitative trait loci influencing exocytotic sympathoadrenal activity (327, 328). CgB is overexpressed in rodent models of genetic (313, 314) as well as acquired (329) hypertension, suggesting augmented sympathoadrenal activity in the pathogenesis of these syn-

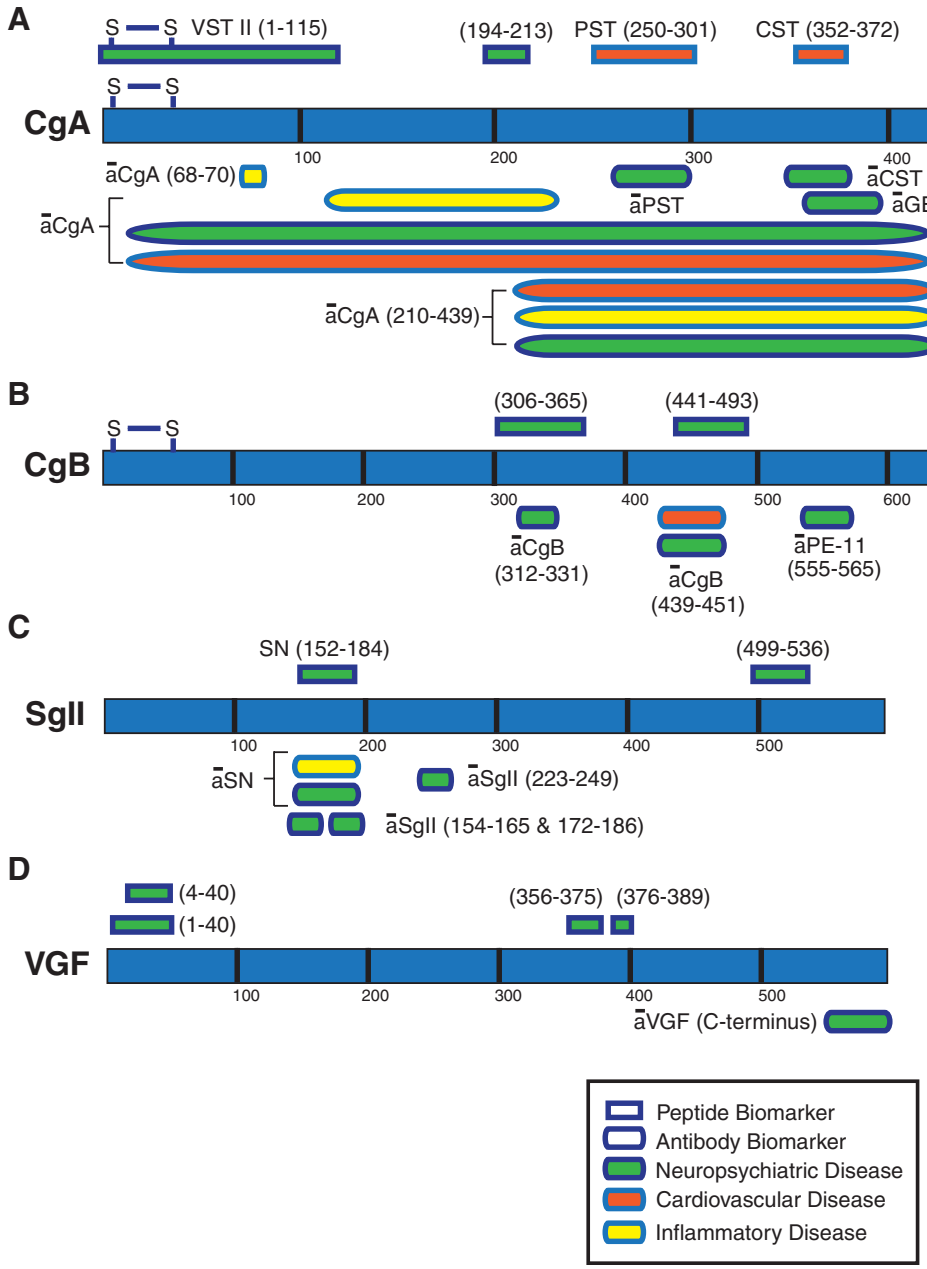

FIG. 7. Utility of granin biomarkers in psychiatric, neurodegenerative, cardiovascular, and inflammatory disease. *Rectangles*, representing peptide fragments of CgA (panel A), CgB (panel B), SgII (panel C), and VGF (panel D) that have been used as biomarkers, are color-coded for disease: psychiatric and neurodegenerative disease (*green*), hypertension and cardiovascular disease (*red*), and inflammatory disease (*yellow*). Granin regions that are recognized by antibodies (labeled ā) used in biomarker studies are denoted by the *similarly colored ellipses*. Note that *ellipses* outnumber *rectangles*, representing the current reliance on antibody-based detection methods and that the application of these granin biomarkers to neurodegenerative and psychiatric disease is comparatively more common. There are fewer biomarker reports for NESP55 (301), proSAAS (354), SgIII (424), and 7B2 (425), so these granins are not included in the illustration. The accompanying Table 5 provides the diseases that these diagnostic biomarkers have been used for and the references describing their application. S-S represents the N-terminal domain containing the disulfide bonded Cys-Cys loop structure.

dromes. Recent twin studies have demonstrated that specific genetic variants (*e.g.*, the CgB A-261T proximal promoter SNP) predict elevated BP and/or BP responses to environmental stress and that these are associated with reduced circulating CgB levels and exaggerated catecholamine secretion (265). Similarly, in women, increased plasma CgB is associated with decreased catecholamine secretion and reduced BP responses to environmental stress. Because of this inverse relationship between CgB expression and either BP or catecholamine secretion, it was hypothesized that experimental disruption of CgB expression would elevate BP. Indeed, targeted ablation of the *Chgb* locus resulted in substantial elevations in both systolic BP (by ~20 mm Hg) and diastolic BP (by ~18 mm Hg) in CgB-KO mice (265), thereby linking CgB and the regulation of BP.

TABLE 5. Utility of granin biomarkers in psychiatric, neurodegenerative, cardiovascular, and inflammatory disease

Biomarker: peptide (aa mature protein) or antibody (region of protein recognized)	Disease and direction of change in biomarker expression (Ref.)
CgA	
VST II (1-115)	↓ AD (353)
CgA (193-213)	↑ MS (357, 358)
PST (250-301)	↑ HTN (315, 316)
CST (352-372)	↓ HTN (243)
CgA	↓ AD (426)
Anti-CgA	⇆ SCZ (217); ⇆ AD (351); ↓ AD (350, 426); ↑ SIRS (335); ↑ HF (253, 317-322, 325); ⇆ cardiac hypertrophy (326)
Anti-CgA (68-70)	↑ RA (427)
Anti-CgA (210-439)	↓ SCZ (219); ↑ MS (357); ↑ IBD (365); ⇆ HF (323)
Anti-PST (250-301)	↓ SCZ (219)
Anti-CST (352-372)	↑ Brain Pick's (345); ↑ brain AD (345); ↓ entorhinal cortex AD (348); ↓ Motor neuron ALS (337, 338); ↑ saliva ALS (339)
Anti-GE25 (b367-391)	↑ SCZ (220); ⇆ MS (428); ⇆ AD 428); ⇆ PD (428)
CgB	
CgB (306-365)	↓ MS (356)
CgB (441-493)	↓ MS (356); ↓ FTD (355)
Anti-CgB (312-331)	↓ MS (356); ↓ SCZ (219); ⇆ thalamus SCZ (218)
Anti-CgB (439-451)	↓ MS (356); ↓ HTN (265); ↑ HF (330); ↑ cardiac muscle HF (330)
Anti-PE11 (555-565)	↓ motor neuron ALS (338); ↓ CA4 and CA3 hippocampus SCZ (221); ⇆ CA1 hippocampus SCZ (221); ↓ cerebral cortex AD (348); ⇆ MS (428); ⇆ AD (428); ⇆ PD (428)
SgII	
SN (152-184)	↓ MS (356)
SgII (499-536)	↓ MDD (224)
Anti-SN (154-186)	↓ motor neuron ALS (338); ↓ cerebral cortex AD (348); ⇆ SCZ (220); ↑ nasal lavage AR (429); ⇆ MS (428); ⇆ AD (428); ⇆ PD (428)
Anti-SgII (154-165) & (172-186)	↓ MS (356)
Anti-SgII (223-249)	⇆ SCZ (219); ⇆ thalamus SCZ (218)
VGF	
VGF (1-40)	↓ FTD (355)
VGF (4-40)	↑ SCZ (224, 225); ↑ MDD (224)
VGF (356-375)	↓ AD (352, 353)
VGF (376-389)	↓ ALS (342, 343)
Anti-VGF (C terminus)	↓ ALS (342, 343)

The peptide and antibody biomarkers illustrated in Fig. 7 are described further here; amino acid (aa) numbers for peptide fragments, shown in *parentheses*, correspond to the mature human granin sequences, as do the regions recognized by antisera (except anti-GE25; b indicates bovine). References for the biomarkers are noted adjacent to the diseases for which they have been employed diagnostically or prognostically. *Arrows* positioned to the left of the disease indicate increased (↑), decreased (↓), or unchanged (⇆) biomarker expression. Neurodegenerative and psychiatric biomarkers were measured in samples of CSF, and the cardiovascular and inflammatory biomarkers were in plasma or serum, unless otherwise noted. Other biomarker measurements were made in human spinal cord motor neurons, brain, nasal lavage, saliva, and cardiac muscle. AD, Alzheimer's disease; AR, allergic rhinitis; FTD, fronto-temporal dementia; HF, heart failure; HTN, hypertension; IBD, inflammatory bowel disease; MDD, major depressive disorder; MS, multiple sclerosis; PD, Parkinson's disease; SCZ, schizophrenia.

Recent studies further support the utility of circulating CgB levels as a biomarker of post-myocardial infarct cardiac failure in human patients, whereas in animal models, CgB mRNA and protein levels are also increased in infarcted and noninfarcted left ventricular myocardium, which is associated with increased levels of circulating CgB (330).

3. SgII biomarkers

Of potential future utility as a biomarker for cardiovascular disease, the SgII-derived peptide SN stimulates migration and proliferation of vascular smooth muscle cells (331) and acts as an endothelial cytokine to promote angiogenesis and vasculogenesis (248, 314). In addition, an association between *SCG2* alleles and hypertension has recently been reported, linking the risk-associated ancestral allele to quantitative regulation of SgII expression through a previously undescribed Phox2-responsive intronic enhancer (332).

C. Inflammatory disease

Granins have been investigated as inflammatory biomarkers, building on the cytokine-like effects of granin peptides on angiogenesis, their antimicrobial activities, and their effects on human neutrophils. Because SN is chemotactic for mononuclear cells, SgII RNA and protein levels in synovium of patients with rheumatoid arthritis (RA) and osteoarthritis (OA) were examined (333). SGII mRNA was expressed in RA and OA synovial fibroblasts, and immunohistochemical staining differences in SGII between RA and OA were noted (333). Processing of CgA and CgB and SgII in the vitreous of patients with diabetic

retinopathy was recently shown to decrease in diabetic retinopathy, resulting in lower levels of a number of granin-derived peptides that function to regulate inflammation (334). Lastly, serum CgA has been used as an early biomarker of disease severity in patients admitted with systemic inflammatory response syndrome (SIRS) (335). Compared with healthy controls, CgA levels were increased in SIRS patients, which correlated with other inflammatory markers and with patient mortality and were elevated to an even greater extent when SIRS was associated with infection (335).

D. Neurodegenerative and neuropsychiatric disease

1. Amyotrophic lateral sclerosis

Low CgA, CgB, and SgII levels and an association with superoxide dismutase 1 (SOD1)-positive intracellular aggregates have been reported in motor neurons of ALS patients (336–338). In addition, salivary CgA is increased in ALS patients, which positively correlates with emotional functioning (339). A common polymorphism ($P^{413}L$) in the CgB gene of ALS patients has recently been identified (340). Individuals having the $P^{413}L$ gene variant had up to 3.5-fold relative risk to develop ALS. A potential mechanism for the increased risk conferred by $P^{413}L$ variant came from a preclinical study that showed that in mice, CgA and CgB interact with and determine the secretion of mutant but not wild-type SOD1 protein, which in turn may trigger microgliosis and neuronal death (341).

A decrease in $VGF_{398-411}$ has also recently been identified as a potential diagnostic biomarker in ALS patients (342). This decrease in CSF VGF progressed with the clinical severity of ALS in both humans and a mouse model (343). Lower VGF immunoreactivity has been identified in lumbar anterior horn from postmortem spinal cord samples of sporadic ALS patients when compared with control patients with other diseases (344). In SOD1 $G^{93}A$ transgenic mice, decreased VGF protein levels in CSF, serum, and spinal cord preceded onset of clinical symptoms, whereas overexpression of full-length VGF in cultured mouse spinal cord neurons protected them against excitotoxic injury (343). In agreement, SUN N8075, a small molecule that exerts a protective effect on ER stress-induced cell death, and potently induces VGF expression, slowed ALS progression and prolonged survival in mutant SOD1 transgenic mouse and rat models (344). The protective effect exerted by SUN N8075 was fully dependent upon its up-regulation of VGF in the spinal cord of ALS mice and rats. Overall, these data suggest that VGF plays a critical role in motor neuron survival and may be a potential new target for drug discovery and development projects aimed at healing this devastating neurological disorder.

2. Dementia

Alzheimer's disease, Pick's disease, and frontotemporal dementia are neurodegenerative disorders that have in common aggregates of straight filaments composed of hyperphosphorylated tau proteins (*i.e.*, tauopathies). Several studies identified increased CgA, SgII, and proSAAS in postmortem filament aggregates in tauopathies (345–349). Decreased CgA levels were measured in the CSF of patients with early-onset Alzheimer's disease (<65 yr old) but not in patients with senile dementia (>65 yr old) (350), although a previous study found no changes in patients' CSF CgA levels (351). More recently, several groups have used proteomics to investigate the potential utility of granins as diagnostic biomarkers for Alzheimer's disease and frontotemporal dementia: 1) $VGF_{378-397}$ fragment is decreased in the CSF of Alzheimer's patients (352, 353); and 2) proSAAS (354), VGF_{26-62} fragment (355), and $CgB_{441-493}$ fragment (355) are decreased in the CSF of patients affected by frontotemporal dementia.

3. Multiple sclerosis

Mattsson *et al.* (356) identified lower levels of two CgB fragments, $CgB_{441-493}$ and $CgB_{306-365}$, and the SgII peptide SN in the CSF of multiple sclerosis patients compared with healthy siblings and nonrelated controls. In contrast, the $CgA_{194-213}$ fragment was increased in the CSF of multiple sclerosis patients when compared with patients suffering from other neurological diseases that lack an inflammatory component (357, 358). Lastly, the $7B2_{125-142}$ fragment was also increased in patients affected by neurological diseases of inflammatory origin compared with clinically isolated syndromes of demyelination (358).

4. Schizophrenia and depression

Schizophrenia is thus far the only psychiatric disease for which CgA- and CgB-encoding genes have been identified as susceptibility loci (261, 359, 360). Despite these genetic association studies, proteomic assays of CSF or postmortem tissue from schizophrenic patients have failed to identify a clear involvement of granin proteins in schizophrenia; 1) increased CgA to SN ratio (220), 2) decreased CgA and CgB but not SgII (219), 3) decreased CgB immunoreactivity in postmortem hippocampi (221), and lastly 4) a negative correlation between CgA, negative symptoms and ventricle to brain ratio in schizophrenic patients (217) have been noted. The future utility of chromogranins as schizophrenia biomarkers will therefore depend on additional investigation.

In contrast, a recent study showed a robust increase in the content of the VGF_{23-62} fragment in CSF samples from first-onset drug-naive schizophrenic patients (224, 225). This important study, having a large sample size, cross-validation groups, and disease-specificity analysis (de-

pression, obsessive-compulsive disorder, and Alzheimer's disease), demonstrated a diagnostic sensitivity of 88% and a specificity of 95% for schizophrenia. The same study also established an increase in the same $VGF_{23–62}$ fragment and a selective decrease in $SgII_{529–566}$ fragment in a small cohort of depressed patients (224). Therefore, it is conceivable that $VGF_{23–62}$ might be associated with an underlying mechanism of both schizophrenia and depression. In contrast, VGF mRNA levels were recently found to be reduced in peripheral leukocytes of drug-free depressed patients as compared with controls and were modulated in response to antidepressant treatment (222).

E. Perspectives. Granin biomarkers: where do we go from here?

Immunostaining of biopsied tissue, RIA, peptide ELISA, unbiased mass spectrometry, and the assessment of gene expression have all been used to identify granins as disease biomarkers (298, 361, 362). However, the diversity of immunoreactive granin-derived peptides that can be obtained from neuroendocrine tumors presents a major diagnostic problem, complicating the use of antigranin antisera in biomarker studies. Antisera can be highly specific for individual peptide epitopes, but these antigenic determinants are likely shared by the full-length granin and a number of processed granin fragments. Nevertheless, commercially available antisera for some of these peptides are widely used as markers for endocrine and neuroendocrine tumors (363, 364). A second issue complicating the utility of granin biomarkers is the specificity of a given biomarker for a particular disease. As discussed in *Section VI.C*, circulating CgA levels are a useful inflammatory biomarker. Recent studies have noted increased plasma CgA levels in inflammatory bowel disease (365), which could complicate the utility of CgA-based assays in the diagnosis of carcinoid or other neuroendocrine tumors in patients with ulcerative colitis or Crohn's disease. Future application of HPLC- and proteomic-based technologies that are able to identify and quantify fragments of granin proteins in plasma, much as is being done in CSF, will likely improve the specificity and overall utility of circulating granins and granin-derived peptides as tumor and disease biomarkers. At least for CSF analysis, combinatorial proteomic assays of multiple biomarkers could soon offer practical diagnostic and prognostic information for degenerative neurological diseases such as ALS. In fact, recent studies suggest that decreased CSF levels of VGF, in combination with other peptide biomarkers, are valuable for ALS diagnosis and perhaps also represent a prognostic biomarker, with lower levels noted before overt neurological disease in mouse ALS models (342, 343). As with other biomarkers, it will be interesting to see whether these encouraging findings translate into medical advances in the diagnosis and treatment of neurodegenerative disease, much as they have in neuroendocrine and endocrine tumor diagnosis and treatment.

VII. Future Directions: The Search for Receptors of Granin-Derived Peptides

As noted in *Section IV*, biological activities have been discovered for a number of granin-derived peptides, but the precise mechanisms of action and cognate receptors remain in most cases elusive. Similarly, genetic evidence is strongly supportive of critical granin functions, but again, the mechanisms by which specific signaling pathways are triggered by granin-derived peptides require further elucidation. This is perhaps the greatest future challenge confronting investigators in the granin field. Studies indicate that specific granin proteins function in granule biogenesis (CgA, CgB, SgII, and SgIII). In some cases, binding interactions with other granins and/or vesicular cholesterol-rich lipids have been clearly defined, and analysis of KO mice has provided support for granin roles in secretory vesicle formation. The same mechanistic progress has not been as easily obtained for granin-derived peptides. As detailed in *Section IV.E*, one CgA-derived peptide, CST, has been demonstrated to function as a noncompetitive nicotinic cholinergic receptor antagonist (76), inhibiting catecholamine release from adrenal chromaffin cells and sympathetic neurons. Findings are compatible with the binding of CST to a site within the nicotinic acetylcholine receptor channel and to a low-affinity site outside the channel (366). Although widely hypothesized to interact with classical G protein-coupled receptors, no published data have directly shown binding of any granin-derived peptides to a known or orphan G protein-coupled receptor. However, in many cases, downstream pathways often associated with G protein signaling are activated in response to peptide treatment. For example, the CgA-derived peptide serpinin increases cAMP levels and adenylate cyclase activity (143), whereas the VGF-derived peptides AQEE-30, TLQP-21, and LQEQ-19 activate MAPK and Erk1/2 kinases (209, 367). In addition, noncompetitive interaction between granin-derived peptides and G protein-coupled receptors, at sites distinct from those that interact with known ligands, is an alternative mechanism. Recently, nontraditional, receptor-independent triggering of signal transduction pathways by granin-derived peptides has been proposed as a possible mechanism of action (368). The CgA-derived peptide VST-1 has membrane-penetrating properties that suggest the potential for receptor-independent, pertussis toxin-sensitive signaling via interaction with the Gαi/o subunit (368). A related model has been

proposed for CST interaction with heterotrimeric G protein in mast cell membranes to modulate histamine release (368). Major future challenges in the granin field will be the identification of receptors or physiologically important binding proteins for granin-derived peptides and further characterization of receptor-dependent and receptor-independent signaling pathways.

VIII. Conclusions

The granin proteins reviewed here share many structural and biochemical features and manifest striking evolutionary conservation. Several members contribute to very diverse functions within the regulated secretory pathway of endocrine and neuronal cells, including granulogenesis and the regulation of peptide processing. After their regulated secretion, granin-derived peptides provide autocrine, paracrine, and endocrine signals, with a range of bioactivities extending from feedback stimulation of LDCV formation to the regulation of hormone and growth factor release. Characterization of KO mouse models in preclinical studies and human genetic analyses suggests important, new functional roles and specific disease associations of granin peptides. Lastly, relatively abundant and selective expression of these secreted proteins in the nervous system and in endocrine and neuroendocrine tissues has led to their increased utility as biomarkers of disease and therapeutic efficacy. The main challenges moving forward will be to identify receptors for granin-derived peptides, elucidate their cellular signaling mechanisms, and apply increasingly powerful proteomic methods, ultimately improving the specificity and sensitivity of granin biomarker measurements in blood and CSF.

Acknowledgments

We thank Drs. Niamh Cawley and Hisatsugu Koshimizu (*Eunice Kennedy Shriver* National Institute of Child Health and Human Development) for critical reading of the manuscript and providing Figs. 5 and 6.

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Research in the authors' laboratories was supported in part by University of Minnesota Medical School (to A.B.); Intramural Research Program of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health (NIH) (to Y.P.L.); NIH (to S.K.M. and S.R.J.S.); and National Alliance for Research on Schizophrenia and Depression, Hope for Depression Foundation, and Diabetes Action Research and Education Foundation (to S.R.J.S.).

Disclosure Summary: The authors have nothing to disclose.

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