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Chromogranins: from Cell Biology to Physiology and Biomedicine

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ISSN 2366-7516 ISSN 2366-7524 (electronic)
UNIPA Springer Series
ISBN 978-3-319-58337-2 ISBN 978-3-319-58338-9 (eBook)
DOI 10.1007/978-3-319-58338-9

Library of Congress Control Number: 2017956128

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

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History and Perspectives

Karen B. Helle

Abstract Research on chromaffin cells dates back to 1856 when the venous outflow of chemical substances from the adrenal medulla into the circulation was first described. The discovery of the chromaffin granules for storage of catecholamines in 1953 was the next major break-through. Soon thereafter the co-storage of catecholamines, ATP and uniquely acidic proteins was established, together making up the isotonic storage complex within elements of the diffuse sympathoadrenal system. The core proteins constitute a family of eight genetically distinct, uniquely acidic proteins, characterized by numerous pairs of basic residues and collectively named granins. A prohormone concept was formulated when the insulin-release inhibiting peptide, pancreastatin, was identified as the mid sequence of porcine chromogranin A. Subsequently, processing resulted in a range of peptides with antifungal and antibacterial potencies, predominantly from chromogranin A, a few from chromogranin B and one from secretogranin II. A wide range of biological activites has since been documented, notably for the chromogranin A –derived peptides, affecting endothelial stability, myocardial contractility, angiogenesis, cell adhesion and tumor progression. A physiological role for full-length chromogranin A and vasostatin-I as circulating stabilizers of endothelial integrity is now evident, while the high circulating levels of chromogranin A in neuroendocrine tumors and inflammatory diseases remain an unsolved and challenging puzzle for future research.

Abbreviations

bCgA	bovine $CgA_{1–431}$
CA	Catecholamines
CgA	Chromogranin A
CgB	Chromogranin B
GE-25	$bCgA_{367–391}$
PN-1	Protease nexin-1
PTH	Parathyroid hormone

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T. Angelone et al. (eds.), *Chromogranins: from Cell Biology to Physiology and Biomedicine*, UNIPA Springer Series, DOI 10.1007/978-3-319-58338-9_1

PTX	Pertussis toxin
SgII	Secretogranin II
VIF	Vasoinhibitory factor – $CgA_{79–113}$
VS-I	Vasostatin I ($CgA_{1–76}$)
VS-II	Vasostatin II ($CgA_{1–113}$)
WE-14	$bCgA_{316–330}$

1 History

1.1 The First Hundred Years of the Chromaffin Cells

Research on chromaffin cells and granins can be traced back to the mid nineteenth century when Vulpian (1856) described the venous outflow of chemical substances from the adrenal medulla into the circulation. Half a century later the strong cardiovascular effects of the adrenomedullary substances (Oliver and Schäfer 1895) led to the chemical identification and synthesis of the first hormones, adrenaline and noradrenaline (Stoltz 1904). We owe the first identification of catecholamines (CA) to the function of the adrenergic neuron to Loewi, who in Loewi 1921 described the so-called Accellerans-Stoff or Sympathin and its stimulating activity on the denervated frog heart. Twenty five years later, Sympathin E was identified as noradrenaline (Von Euler 1946).

1.2 The First Decade of the Chromaffin Granules

The discovery of the subcellular organelles responsible for the storage of CA in the adrenomedullary chromaffin cells, i.e. the chromaffin granules, was a major break-through (Blaschko and Welsch 1953, Hillarp et al. 1953). Soon thereafter the chromaffin granules were shown to be electron-dense, membrane-limited granules of 150–300 mμ diameter (Lever 1955, Welzstein 1957, Hagen and Barnett 1960, Coupland 1968). In parallel, the vesicles related to the storage of noradrenaline in the adrenergic fibres (Von Euler and Hillarp 1956, Von Euler 1958, Dahlstrøm 1966) were demonstrated to be smaller and of varying size and electron density both in the axons and in the terminals (De Robertis and Pellegrino de Iraldi 1961). Biochemical studies, on the other hand, revealed that both types of organelles bore a number of similarities, such as storing the respective CA together with the energy-rich nucleotide ATP in a molar ratio of CA: ATP of close to 4:1 in the adrenomedullary (Blaschko et al. 1956, Falck et al. 1956) and of 5:1 in the adrenergic nerve granules (Schümann 1958; Banks et al. 1969). Moreover, in the adrenomedullary chromaffin cells these low molecular weight

constituents were stored intragranularly at concentrations of about 0.55 and 0.13 M for CA and ATP respectively, i.e. strongly hypertonic if osmotically active. This phenomenon led Hillarp in 1959 to the postulation of a third component involved in the storage complex, possibly a protein, which could be responsible for holding CA and ATP in an isotonic, non-diffusible form until discharge from the stimulated cell.

1.3 The First Thirty Five Years of the Granins

The search for a specific macromolecule involved in the isotonic retention of CA and ATP within the storage organelles was immediately directed to the core proteins in the bovine adrenomedullary chromaffin granules (Helle 1966a, Smith and Winkler 1967, Smith and Kirshner 1967). By means of an immunological identification method (Helle 1966b) it was established that the enzymatically inactive protein, subsequently named chromogranin (Blaschko et al. 1967), was exocytotically discharged from the stimulated adrenal gland in parallel with the co-stored CA and ATP both in vitro (Banks and Helle 1965) and in vivo (Blaschko et al. 1967). Due to the easy access from local slaughterhouses the bovine adrenals soon became a convenient source of chromaffin cells and chromaffin granules (Smith and Winkler 1967), notably for research on the structural, chemical and functional properties of the family of chromogranins, i.e. the granins (Huttner et al. 1991; Winkler and Fischer-Colbrie 1992).

1.3.1 Glucose Homeostasis, Pancreastatin and the Prohormone Concept

The first chromogranin A (CgA) peptide to be recognized for its regulatory potency was named pancreastatin due to its ability to inhibit the rapid phase of insulin release from the glucose-stimulated porcine pancreas (Tatemoto et al. 1986; Efendic et al. 1987). When identified as the mid-section of porcine and human CgA (Huttner and Benedum 1987; Konecki et al. 1987), a novel concept was coined, namely of the granins as putative prohormones for biologically active peptides with regulating potentials (Eiden 1987). Subsequently, pancreastatin was shown to be involved as a regulator of insulin action not only of glucose but also of lipid and protein metabolism (Sanchez-Margalet and Gonzalez-Yanes 1998). In rat hepatoma cells also the cell growth was inhibited, depending on the availability of nitric oxide (NO) production (Sanchez-Margalet et al. 2001). The accumulated literature supports the original observation of pancreastatin as an anti-insulin agent, impairing glucose homeostasis by diminishing insulin sensitivity (see review by Valicherla et al. 2013).

1.3.2 Calcium Homeostasis and the N-Terminus of CgA

In the parathyroid gland CgA was originally described as parathyroid secretory protein-I (Cohn et al. 1981), co-secreted from the gland with the parathyroid hormone (PTH), i. e. the primary regulator of serum calcium concentrations. Peptides containing the N-terminal sequence of CgA ($CgA_{1–76}$) inhibit PTH-secretion as effective as high physiological concentrations of calcium (Fasciotto et al. 1990). Pancreastatin ($bCgA_{248–293}$) and parastatin ($bCgA_{347–419}$) have also been shown to inhibit PTH secretion, but not yet detected in the effluents from the parathyroid cells in vivo. On the other hand, $CgA_{1–76}$ was detected both in the medium of cultured parathyroid cells (Angeletti et al. 2000) and in the adrenomedullary effluents (Metz-Boutigue et al. 1993). A binding to a 78 kDa protein was identified on the parathyroid cell surface, and the blockade by pertussis toxin indicates a G-protein-coupled receptor. Moreover, the loop sequence $CgA_{16–40}$ was required for inhibition of PTH secretion (Angeletti et al. 1996). Thus, inhibition of PTH secretion by CgA predominantly involves $CgA_{1–76}$, occuring either by an autocrine mechanism or via the circulating concentrations of the processed peptide.

1.4 The Granins and their Derived Peptides

Detailed investigations of the eight members of the granin family, i.e. CgA, chromogranin B (CgB), secretogranin II (SgII) and secretogranins III-VII, have since documented that these proteins are widely distributed in distinct patterns within the diffuse neuroendocrine system of vertebrates (Helle 2004). Stimuli for release of the granins derive from a wide range of environmental and intrinsic paths, raising the concentrations of the intact prohormones and processed peptides in the extracellular space and ultimately in the circulation. The degree of processing is extensive in the adrenomedullary storage granules (Metz-Boutigue et al. 1993; Strub et al. 1995) and gives rise to a wide range of peptides with a broad spectrum of biological potencies (Helle and Angeletti 1994). The peptides derived from CgA are the vasostatins I and II, chromofungin, chromacin, pancreastatin, catestatin, WE 14, chromostatin, GE25 and parastatin and, in addition, the two most resent arrivals on the scene, serpinin ($CgA_{403–428}$, Koshimizu et al. 2010) and the vasoconstriction-inhibiting factor (VIF, $CgA_{79–113}$, Salem et al. 2015). Vasostatin I (VS-I, $CgA_{1–76}$) and bovine catestatin ($bCgA_{344–364}$) were discovered and named according to their respective inhibitory potencies, on vasodilation (Aardal and Helle 1992) and on CA secretion (Mahata et al. 1997). Since then, notably VS-I and catestatin have been shown to be involved in regulation of a wide range of mechanisms, such as endothelial permeability, angiogenesis, myocardial contractility and innate immunity, however, in many tissue exhibiting oppositely directed activities (Helle et al. 2007; Helle 2010a, b; Mahata et al. 2010).

Peptides derived from CgB, being more extensively processed than CgA in most systems and species (Strub et al. 1995), may have specific regulatory functions yet to be unravelled. SgII, on the other hand, serves a prohormone for only one conspicuously active principle, secretoneurin (Kirchmair et al. 1993, Trudeau et al. 2012), nevertheless engaged in a wide range of modulating activities related to tissue repair (Helle 2010a). Stimulated polymorphonuclear neutrophils, when accumulated in response to invading microorganism, tissue inflammation and at sites of mechanical injury, represent a non-neuroendocrine source of CgA peptides that may affect a wide range of cells involved in inflammatory responses (Lugardon et al. 2000; Zhang et al. 2009). Among them we find the vascular endothelium, the endocardium and the epithelial cells, other leucocytes, fibroblasts, cardiomyocytes, vascular and intestinal smooth muscle cells (Helle et al. 2007; Helle 2010a, b). Taken together, the release of CgA-derived peptides from gland cells, nerve terminals and immunocytes would contribute to autocrine or paracrine modulations locally while endocrine effects would result from their subsequent overflow to the circulation.

1.4.1 The Antimicrobial Peptides and Innate Immunity

Antimicrobial activities of peptides derived from the matrix of secretory granules in the bovine adrenal medulla were first reported by Metz-Boutigue and colleagues in 1998. The first three peptides found to inhibit bacteria and fungal growth were derived from the N-terminal domain of CgA (VS-I), the C-terminal end of CgB (secretolytin) and the biphosphorylated C-terminal peptide of proenkephalin-A (enkelytin). These peptides are active in a diverse range of organisms, including prokaryotes, bivalves, frogs and mammals, suggesting an important role in innate immunity, a mechanism shared by all vertebrates and present at birth as an evolutionary ancient defence mechanism (Hoffmann et al. 1999; Metz-Boutigue et al. 2000). Another CgA peptide, catestatin, derived from CgA in keratinocytes, also possess antimicrobial activity against gram-positive and gram-negative bacteria, yeast and fungi, is active notably against skin pathogens and increases in skin in response to injury and infection (Radek et al. 2008). So far, no antimicrobial activity has been assigned to SN.

The innate immunity, independent of the adaptive immune responses, is used by vertebrates as a means for short term protection against pathogenic microorganisms. The need for new antimicrobial agents is now rapidly rising due to the fast growing number of antibiotica-resistant bacteria. Accordingly, the interest in antibacterial granin-derived peptides has grown exponentially. Their therapeutic potentials are now under intensive elucidation in immunodeficient patients, in chemotherapy, in organ grafting, and against antibiotica-resistant bacterial infections (Shooshtarizodeh et al. 2010).