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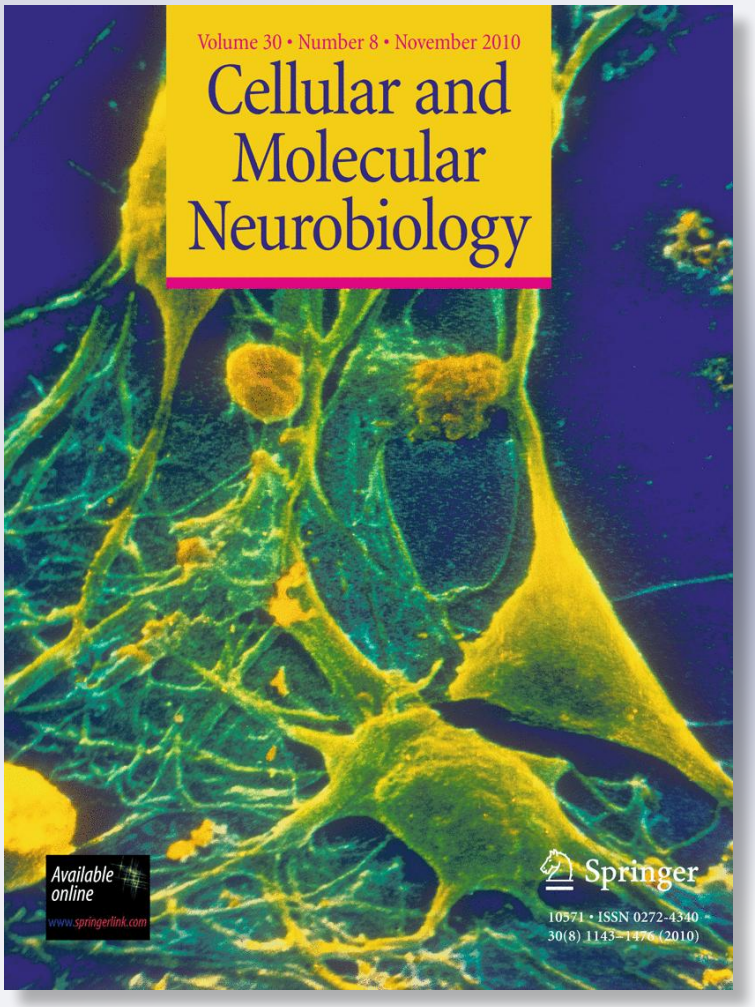

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REVIEW PAPER

Chromogranins A and B as Regulators of Vesicle Cargo and Exocytosis

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Abstract Chromogranins (Cgs) are acidic proteins that have been implicated in several physiological processes such as vesicle sorting, the production of bioactive peptides and the accumulation of soluble species inside large dense core vesicles (LDCV). They constitute the main protein component in the vesicular matrix of LDCV. This latter characteristic of Cgs accounts for the ability of vesicles to concentrate catecholamines and Ca^{2+}. It is likely that Cgs are behind the delay in the neurotransmitter exit towards the extracellular milieu after vesicle fusion, due to their low affinity and high capacity to bind solutes present inside LDCV. The recent availability of mouse strains lacking Cgs, combined with the arrival of several techniques for the direct monitoring of exocytosis, have helped to expand our knowledge about the mechanisms used by granins to concentrate catecholamines and Ca^{2+} in LDCV, and how they affect the kinetics of exocytosis. We will discuss the roles of Cgs A and B in maintaining the intravesicular environment of secretory vesicles and in exocytosis, bringing together the most recent findings from adrenal chromaffin cells.

Keywords Amperometry · Catecholamines · Chromaffin · Secretion

Abbreviations

Cgs	Chromogranins
CgA	Chromogranin A
CgB	Chromogranin B
LDCV	Large dense core vesicles
SgII	Secretogranin II

By the end of the 1950's an acidic protein was identified in large dense core vesicles (LDCV)—chromaffin granules—of chromaffin cells which was called chromogranin A (CgA) and turned out to be the first member of a family often denominated granins (Hillarp 1959). The presence of this water-soluble glycoprotein that co-secretes with catecholamines from adrenomedullary cells also provided the first direct evidence of exocytosis on the basis that these proteins could not be released in any other way (Banks and Helle 1965). This family has since grown to also include chromogranin B (CgB or SgI), secretogranin II (SgII or CgC), SgIII (or 1B1075), SgIV (or HISL-19), SgV (or 7B2), SgVI (or NESP55), SgVII (or VGF), and pro-SAAS, which are also found in many other tissues such as the pituitary gland and islets of Langerhans, as well as in brain neurons (Taupenot et al. 2003; Helle 2010; Zhao et al. 2009; Montero-Hadjadje et al. 2009).

The first function assigned to Cgs was to help to concentrate catecholamines and other solutes inside the LDCV of secretory cells. Hence, the ability of Cgs to bind catecholamines is thought to reduce osmotic pressure and prevent the swelling vesicles from bursting (Helle et al. 1985). This hypothesis involving the adsorption of soluble species to Cgs received strong support when amperometric recording of single exocytotic events from adrenal chromaffin cells was first initiated (Wightman et al. 1991).

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Unexpectedly, these experiments showed that catecholamines from fused LDCV are released at a very slow rate (Wightman et al. 1991), proof that a functional matrix retains these catecholamines. In fact, it was proposed that a similar situation also occurs for other solutes, like Ca^{2+} and ATP, for a more extensive review about the role of LDCV as an active Ca^{2+} reservoir see a recent publication (Yoo 2010).

In addition, Cgs have been implicated in the biogenesis of the LDCV, as well as in the sorting mechanisms that occur in these vesicles (Kim et al. 2001; 2005; Huh et al. 2003; Gerdes and Glombik 2000; Glombik et al. 1999; Courel et al. 2008). Moreover, Cgs are also considered as pro-hormones as they constitute a source of bioactive peptides, reviewed in Montero-Hadjadje et al. (2008), Taupenot et al. (2003), and Zhao et al. (2009). These proteins contain multiple dibasic cleavage sites, although it is likely that most of these processing systems are not active in situ due to the inhibition of endopeptidases by the elevated vesicle concentration of catecholamines (Wolkersdorfer et al. 1996) or the cross inhibition by other granins (Koshimizu et al. 2010). Furthermore, Cgs currently serve as tumor markers (O'Connor et al. 1983; Conlon 2010) and as a sign of a worsening prognosis for some kind of cancers (Gregorc et al. 2007). Recently, Cgs were proposed to be directly involved in the development of some neurological diseases such as schizophrenia (Zanner et al. 2002), epilepsy (Mahata et al. 1992), Parkinson's (Nishimura et al. 1994) and Alzheimer's disease (Munoz 1991; Marksteiner et al. 2000), as well as amyotrophic lateral sclerosis (Schrott-Fischer et al. 2009).

In this review, we will focus our attention on the most relevant issues related to the participation of Cgs in amine accumulation in the LDCV and in their release, principally in adrenal chromaffin cells.

Extremely High Concentrations of Solutes are Present in LDCV

Chromaffins are essentially secretory cells that contain around 15,000 LDCV; however, only a small fraction is suitable for immediate release (Trifaro et al. 1997; Heinemann et al. 1994). The sympatho-adrenal response to stress requires the secretion of large amounts of catecholamines and peptides into the bloodstream, which is possible due to the surprisingly high concentration of solutes inside the LDCV.

Vesicular catecholamines are estimated to be 0.5–1 M depending on the animal species studied (Winkler and Westhead 1980; Pihel et al. 1994; Albillos et al. 1997; Montesinos et al. 2008). Vesicles also contain large amounts of ATP (Weber et al. 1983), of Ca^{2+} (Bulenda and Gratzl 1985), of ascorbate (Terland and Flatmark 1975), as well as unquantified amounts of biopeptides, other nucleotides, dopamine-β-hydroxylase, and H^+, all of which together with Cgs forms a condensed matrix at an acidic pH of $\approx$5.5 in the vesicles. The theoretical osmolarity of this mixture would be around 1,500 mOsm. The functional aggregation of soluble species is necessary to maintain the homeostasis of LDCV and avoid their disruption due to osmotic stress. As CgA and CgB are quantitatively the most important granins in the LDCV, they are the main candidates to facilitate the condensation of soluble species required to generate the dense core (Helle et al. 1985). This functional matrix is probably the same as that observed in electron microscopy images of LDCV as an electrodense core. Indeed, the vesicular content of Cgs in bovine chromaffin LDCV is estimated to be $\approx$1.8 mM of CgA, $\approx$200 μM of CgB, and $\approx$30 μM of SgII (Yoo 2010), although their relative amounts may also vary depending on the species.

Chromogranins are currently considered as a saturable high capacity and low affinity buffer. For instance, CgA binds 32 mol adrenaline per mol with a Kd of 2.1 mM (Videen et al. 1992), and Cgs also bind Ca^{2+} $\approx$50 mol per mol of Cg with a Kd of 1.5 to 4 mM depending on the type of granin (Yoo and Albanesi 1991; Yoo 2010). The ability of CgA and CgB to interact with each other to form dimers or hetero-tetramers has been studied to explore the interaction of Cgs with Ca^{2+} (Yoo 1996; Yoo and Albanesi 1991). However, a similar interaction with soluble species such as catecholamines and ATP is likely to occur as the presence of multiple dibasic groups in their structure increases their ability to concentrate solutes (Yoo and Albanesi 1990; Yoo 1996; Park et al. 2002).

The intravesicular compounds can be divided into two major groups depending on their capacity to move across the vesicle's membrane. Hence, amines, ascorbate, H^+, Ca^{2+} and ATP are "mobile components" that are able to move across the vesicle membrane, whereas Cgs and other proteins like enzymes are considered as "immobile components" as they cannot easily leave the vesicles. All mobile compounds of the vesicular cocktail are in equilibrium with the cytosol and the matrix, and as such, they are all the main candidates to be involved in the regulation of exocytosis since changes in any one of these species will affect the others.

The Release of Catecholamines from the LDCV During Exocytosis is Unexpectedly Slow

When single LDCV fusion events occur the release of adrenaline is two to three orders of magnitude slower than that predicted by the diffusion coefficient of catecholamines in aqueous media (Gerhardt and Adams 1982;

Hafez et al. 2005). Two mechanisms could explain why catecholamines are retained inside the fused vesicle:

- The diameter of the fusion pore that could limit the free escape of soluble species from the vesicle and,
- The slow diffusion of solutes from the LDCV matrix.

Measurements obtained with patch-amperometry, a technique that combines amperometry with cell-attached capacitance, revealed that the arrival of catecholamines to the carbon fiber electrode was still delayed even when the fusion pore was dilated (Albillos et al. 1997; Montesinos et al. 2008). This suggests the direct involvement of the vesicle matrix in the slow release of amines observed once vesicle fusion has taken place.

Some indirect approaches also connect the slow release to the nature of the vesicle's protein matrix. For instance, exocytosis is largely delayed in the presence of cross-linking agents like glutaraldehyde or formaldehyde that should freeze the dissociation of catecholamines from Cgs (Borges et al. 2000). Moreover, in experiments on chromaffin cells cultured in astrocyte conditioned media, the phenotype of the chromaffin cells switches to a neuronal-like form observed with electron microscopy that exhibited many small vesicles containing little dense material (Ardiles et al. 2006). Using amperometry, these authors observed a drastically accelerated exocytosis suggesting a close relationship between the presence of vesicular matrix and the kinetics of exocytosis.

Catecholamine Exocytosis from CgA-KO Chromaffin Cells

The arrival of a mouse lacking CgA was recently achieved by the targeted ablation of the CgA gene in mice to generate CgA-KO animals that were viable and fertile in homozygosis (Mahapatra et al. 2005; Hendy et al. 2006). This provided the first opportunity to study exocytosis in the absence of one of the "immobile components". Although the effects that the lack of CgA produced should be taken with care due to the partial compensation by the overexpression of CgB (Fig. 1a, b), the redistribution of Cgs produced drastic effects in the storage and release of catecholamines from the LDCV of adrenal chromaffin cells.

Although CgA was suggested to be essential for granule biogenesis and sorting, chromaffin cells from CgA-KO can still release adrenaline by exocytosis. Using amperometry as the method of quantification we concluded that cells from CgA-KO mice released $\approx$40% less catecholamines after a depolarizing stimulus (Fig. 2b), and that the number of spikes from the WT and CgA-KO cells was similar over a 2 min recording (Fig. 2a). In addition, the net content of catecholamines per quantum (Q) was reduced by 34%

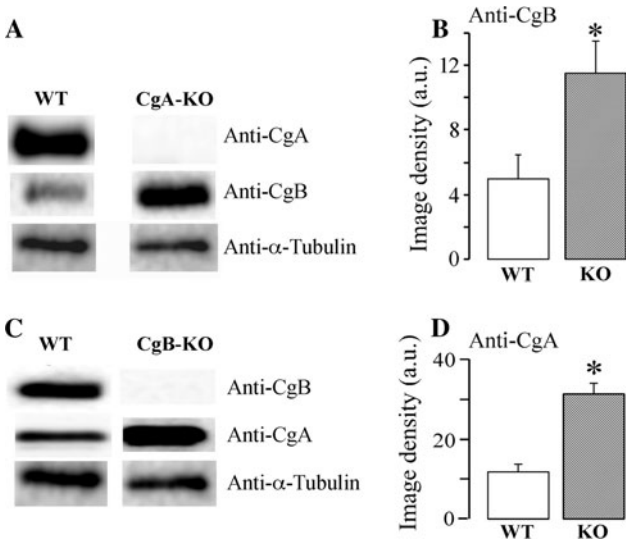


Fig. 1 The lack of CgA promotes the overexpression of CgB and vice versa. **a** Western-blots of the adrenal medulla confirming the lack in CgA and the overexpression of CgB. **b** Quantification of CgB in the WT and CgA-KO as the average from 3 wells containing 4 different medullas (means ± SEM). **c** As in **a** but showing the overexpression of CgA in CgB-KO mouse. **d** Quantification of CgA in the WT and CgB-KO as the average from 3 wells containing 4 different medullas (means ± SEM). The gels' densities are expressed in arbitrary units (a.u.), α-tubulin was used as an internal control. * $P < 0.05$; ** $P < 0.01$ Mann–Whitney test. Modified from Montesinos et al. (2008) and Diaz-Vera et al. (2010)

(Fig. 2c) and the kinetic analysis of secretory spikes showed that exocytosis occurred faster in CgA-KO cells, these kinetic changes mainly affect the last part of spikes (Fig. 3). Taken together, these data indicate that the matrix of the LDCV without CgA is less capable of concentrating and retaining catecholamines thereby causing exocytosis to occur faster (Montesinos et al. 2008).

When chromaffin cells were overloaded with the catecholamine precursor L-DOPA, a second important consequence of the lack in CgA was revealed. L-DOPA crosses the chromaffin cell membranes and it is rapidly converted into dopamine, which is usually taken up by the LDCV where the dopamine is converted in noradrenaline by dopamine-β-hydroxylase. The usual effect of L-DOPA incubation is a notable increase in the vesicular content of catecholamines (Colliver et al. 2000; Sombers et al. 2007; Gong et al. 2003) as observed in cells from WT mice. However, the LDCV of chromaffin cells from CgA-KO could not take up more amines.

We performed experiments using intracellular electrochemistry in the presence of the monoamino oxidase inhibitor pargyline to check whether this impediment to storing more catecholamines was derived from a reduction in the availability of cytosolic catecholamines. This technique is a modified version of patch-amperometry using the whole-cell configuration, thereby allowing a carbon fiber

electrode to be brought into contact with the cytosolic medium. Chromaffin cells from CgA-KO animals had less free catecholamines than their WT counterparts. However, there was a marked increase of free cytosolic amines in the KO mice after incubation with L-DOPA (100 μM during 90 min) when compared with WT mice. Hence, the newly synthesized amines appear to be unable to enter the LDCV because their saturated matrix left no room for new catecholamines (Montesinos et al. 2008).

Using patch-amperometry in the cell-attached configuration to simultaneously monitor the vesicle size (capacitance) and the release of catecholamines from the same vesicle (amperometry) we studied, in more detail, the storage and release properties of the LDCV lacking CgA Our data revealed that the vesicular concentration of catecholamines drops from 870 mM in WT to 530 mM in the CgA-KO. In addition, the lack of CgA causes a delay in the release of catecholamines from the moment that fusion occurs to the arrival of catecholamines at the amperometric detector, suggesting a direct role of CgA in the swelling of the LDCV upon fusion.

Our data indicate that the LDCV from chromaffin cells lacking CgA have a much weaker capacity to accumulate catecholamines, even when considering the overexpression of CgB. Having demonstrated a role for CgA in exocytosis, we concentrated our efforts on the other major chromogranin, CgB.

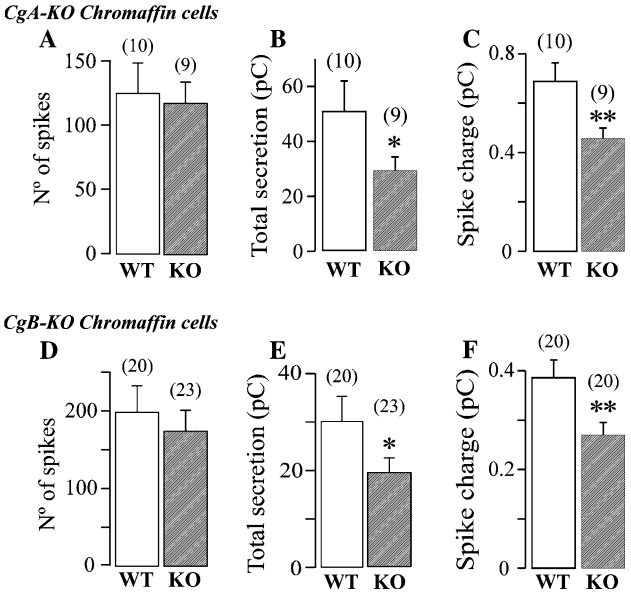
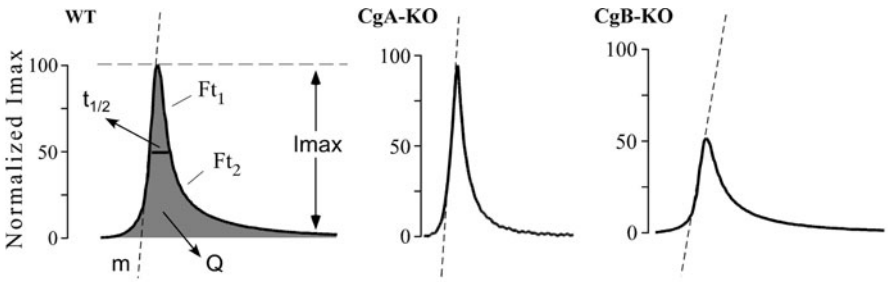


Fig. 2 Secretory characteristics of the Cgs-KO chromaffin cells. Data from amperometry experiments carried out in CgA-KO- (**a**–**c**), and from CgB-KO-cells (**d**–**e**). Secretory spikes were counted in the first 2 min after $BaCl_2$ stimulation (**a**, **d**). Total secretion was measured by integration of these spikes (**b**, **e**). The charges of individual spikes from CgA-KO and CgB-KO cells compared with their own controls (WT) are shown in panels (**c**) and (**f**), values expressed in pCoulombs. Means ± SEM, the number of cells analyzed are shown in *brackets* * $P < 0.05$; ** $P < 0.01$ Mann–Whitney test. Modified from Montesinos et al. (2008) and Diaz-Vera et al. (2010)

Catecholamine Exocytosis from CgB-KO Chromaffin Cells

The first mouse strain lacking CgB has recently become available. These mice developed a phenotype of hypertension (Zhang et al. 2009) and intolerance to glucose



	Imax	Q	$t_{1/2}$	m	Ft_1	Ft_2
CgA-KO	-2%	-34%**	-33%*	-7%	-27%*	-19%*
CgB-KO	-45%*	-31%*	+5%	-48%**	+28%	+22%

Fig. 3 Kinetic profiles of amperometric spikes from CgA- and CgB-KO chromaffin cells. Traces illustrate the kinetic changes observed in exocytosis from the cells lacking CgA or CgB. The spikes were constructed by averaging spikes from WT, CgA-KO and CgB-KO and normalized to the I_{max} (100%) of their own control cells. *Discontinuous lines* show the ascending slopes obtained by the linear fit of the 25–75% segment of the ascending portion of the spikes. The table shows the changes of amperometric parameters normalized to their own control cells from WT animals. * $P < 0.05$; ** $P < 0.01$ Mann–Whitney Modified from Diaz-Vera et al. (2010)

(Obermuller et al. 2010). In contrast to the CgA-KO mouse, immuno-histochemistry and western blotting confirmed the absence of CgB and the overexpression of CgA (Fig. 1c, d). We carried out experiments to examine the secretory characteristics of chromaffin cells in these mice in a similar way to the one followed in the CgA-KO strain.

As in the case of CgA, CgB has also been proposed as a critical factor in the genesis and sorting of LDCV (Glombik et al. 1999; Kromer et al. 1998; Natori and Huttner 1996) and if this were true, the lack of this protein should result in the absence of LDCV. However, amperometry shows that chromaffin cells from CgB-KO mice can maintain the release of catecholamines by exocytosis. Moreover, chromaffin cells from WT and CgB-KO mice exhibited similar secretory patterns with no differences in the number of spikes (Fig. 2d). However, the total amount of catecholamines released was 33% lower (Fig. 2e), roughly coinciding with the amount released per quanta (Fig. 2f). A careful analysis of the kinetic properties of secretory spikes revealed the slowing of exocytosis that, contrary to that observed with the CgA-KO mouse, largely affected the first (ascending) part of the spikes (Diaz-Vera et al. 2010) (Fig. 3).

L-DOPA overloading showed that LDCV cannot take up more catecholamines and that the excess of amines that is unable to enter the vesicles remains in the cytosol. From these data, we can conclude that in the absence of CgB, CgA was overexpressed in the adrenomedullary tissues of the CgB-KO mice. Moreover, the cells were capable of releasing catecholamines with a similar frequency of events as the WT cells, although exocytosis exhibited a marked reduction in terms of the quantal size of the secretory packages, which produced an important fall in the total secretory response of chromaffin cells. Finally, the kinetics of exocytosis is affected in the initial part of the spikes (Fig. 3) and the LDCV from CgB-KO cells are unable to take up more catecholamines even though they were abundant in the cytosol, suggesting a saturation of their storage mechanisms.

In order to determine whether other granins, in addition to CgA, could fulfill the role of CgB in forming the dense matrix, our group performed a proteomic analysis of the enriched LDCV fraction from mouse adrenal medullas (Diaz-Vera et al. 2010). This kind of study on the LDCV of mouse chromaffin cells was not easy to perform due to the minute amount of adrenomedullary material available to obtain a purified fraction of vesicles, requiring a minimum of 28–30 adrenal glands for a single 2D gel. There were no significant changes in the amount of SgII expressed in the LDCV fraction and in addition, the expression of all the other granins remained constant. However, the exact quantification of Cgs was difficult because they were usually accompanied by fragments generated by intravesicular proteolysis (Lee and Hook 2009). The lack of CgB produced changes in the expression of other proteins and when we combed the areas of the 2D gels where other granins should be present, none of them were overexpressed. These proteins can be roughly grouped into: typical LDCV proteins; proteins classically associated to mitochondria; those involved in protein folding; cytoskeletal; and "unexpected proteins". One may speculate that the CgB-KO mouse lacks several CgB-derived peptides, and that these animals could therefore release more CgA-derived ones. An attractive hypothesis is that some of the changes observed in the metabolic glucidic or lipidic profile could be originated by the alteration of granule proteins.

Our study revealed important amounts of fibrinogen, for which the three chains (α, β, and γ) were only present in the LDCV of the CgB-KO. Besides its crucial role in clot formation, fibrinogen has recently been associated with the sorting of constitutive vesicles (Gerdes and Glombik 1999). By weight, no other protein seems to be capable of fulfilling the role of Cgs as a matrix-condenser for soluble intravesicular components (Diaz-Vera et al. 2010).

The new data provide the first direct evidence implicating CgB in vesicular storage and in the exocytotic release of CA. We have also demonstrated that the frequency of secretory events is maintained, even in the complete absence of CgA or CgB. The lack of Cgs impairs vesicular accumulation of CA in CgB-KO cells, suggesting saturation of the capacity of the vesicles to store more catecholamines but not any effect on the biogenesis of LDCV. A protein analysis of the secretory vesicle fraction has shown the compensative overexpression of one Cg when the other is absent. Unexpectedly, other proteins apparently unrelated to the secretory process were only present in the adrenomedullary tissue of KO animals.

Concluding Remarks

Since their discovery, Cgs have captivated the attention of scientists, the functions originally proposed for these proteins have expanded into a much wider range of roles including granule biogenesis and sorting, thereby serving as a source of bioactive peptides or as tumor markers, and as proteins with pathophysiological implications in degenerative diseases of the CNS. This review addresses the functional role of CgA and CgB in regulating exocytosis from the LDCV. The generation of mice strains lacking either CgA or CgB has opened up the possibility of studying the role of immobile components. Granulogenesis is not affected by the lack of either granin, although chromaffin cells release significantly less catecholamines *per quantum* and the kinetics of their release differ

substantially. In conclusion, Cgs are highly efficient systems directly involved in monoamine accumulation and in the kinetics of exocytosis from the LDCV. New data point to a fine regulation of the concentration of the "mobile components" of the LDCV by Cgs, where each protein exhibited clear differences in their ability to bind and release neurotransmitters.

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References

Albillos A, Dernick G, Horstmann H, Almers W, Alvarez de Toledo G, Lindau M (1997) The exocytotic event in chromaffin cells revealed by patch amperometry. Nature 389:509–512

Ardiles AO, Maripillan J, Lagos VL, Toro R, Mora IG, Villarroel L, Ales E, Borges R, Cardenas AM (2006) A rapid exocytosis mode in chromaffin cells with a neuronal phenotype. J Neurochem 99:29–41

Banks P, Helle K (1965) The release of protein from the stimulated adrenal medulla. Biochem J 97:40C–41C

Borges R, Machado JD, Alonso C, Brioso MA, Gomez JF (2000) Functional role of chromogranins. The intragranular matrix in the last phase of exocytosis. Adv Exp Med Biol 482:69–81

Bulenda D, Gratzl M (1985) Matrix free Ca^{2+} in isolated chromaffin vesicles. Biochemistry 24:7760–7765

Colliver TL, Pyott SJ, Achalabun M, Ewing AG (2000) VMAT-mediated changes in quantal size and vesicular volume. J Neurosci 20:5276–5282

Conlon JM (2010) Granin-derived peptides as diagnostic and prognostic markers for endocrine tumors. Regul Pept. doi:10.1016/j.regpep.2009.11.013

Courel M, Vasquez MS, Hook VY, Mahata SK, Taupenot L (2008) Sorting of the neuroendocrine secretory protein Secretogranin II into the regulated secretory pathway: role of N- and C-terminal alpha-helical domains. J Biol Chem 283:11807–11822

Diaz-Vera J, Morales YG, Hernandez-Fernaud J, Camacho M, Montesinos MS, Calegari F, Huttner WB, Borges R, Machado JD (2010) Chromogranin B gene ablation reduces the catecholamine cargo and decelerates exocytosis in chromaffin secretory vesicles. J Neurosci 30:950–957

Gerdes HH, Glombik MM (1999) Signal-mediated sorting to the regulated pathway of protein secretion. Ann Anat 181:447–453

Gerdes HH, Glombik MM (2000) Signal-mediated sorting of chromogranins to secretory granules. Adv Exp Med Biol 482:41–54

Gerhardt G, Adams RN (1982) Determination of diffusion-coefficients by flow-injection analysis. Anal Chem 54:2618–2620

Glombik MM, Kromer A, Salm T, Huttner WB, Gerdes HH (1999) The disulfide-bonded loop of chromogranin B mediates membrane binding and directs sorting from the trans-Golgi network to secretory granules. EMBO J 18:1059–1070

Gong LW, Hafez I, Alvarez de Toledo G, Lindau M (2003) Secretory vesicles membrane area is regulated in tandem with quantal size in chromaffin cells. J Neurosci 23:7917–7921

Gregorc V, Spreafico A, Floriani I, Colombo B, Ludovini V, Pistola L, Bellezza G, Vigano MG, Villa E, Corti A (2007) Prognostic value of circulating chromogranin A and soluble tumor necrosis factor receptors in advanced nonsmall cell lung cancer. Cancer 110:845–853

Hafez I, Kisler K, Berberian K, Dernick G, Valero V, Yong MG, Craighead HG, Lindau M (2005) Electrochemical imaging of fusion pore openings by electrochemical detector arrays. Proc Natl Acad Sci USA 102:13879–13884

Heinemann C, Chow RH, Neher E, Zucker RS (1994) Kinetics of the secretory response in bovine chromaffin cells following flash photolysis of caged Ca^{2+}. Biophys J 67:2546–2557

Helle KB (2010) Regulatory peptides from chromogranin A and secretogranin II: putative modulators of cells and tissues involved in inflammatory conditions. Regul Pept. doi:10.1016/j.regpep.2009.09.009

Helle KB, Reed RK, Pihl KE, Serck-Hanssen G (1985) Osmotic properties of the chromogranins and relation to osmotic pressure in catecholamine storage granules. Acta Physiol Scand 123:21–33

Hendy GN, Li T, Girard M, Feldstein RC, Mulay S, Desjardins R, Day R, Karaplis AC, Tremblay ML, Canaff L (2006) Targeted ablation of the chromogranin a (Chga) gene: normal neuroendocrine dense-core secretory granules and increased expression of other granins. Mol Endocrinol 20:1935–1947

Hillarp NA (1959) Further observations on the state of the catechol amines stored in the adrenal medullary granules. Acta Physiol Scand 47:271–279

Huh YH, Jeon SH, Yoo SH (2003) Chromogranin B-induced secretory granule biogenesis: comparison with the similar role of chromogranin A. J Biol Chem 278:40581–40589

Kim T, Tao-Cheng JH, Eiden LE, Loh YP (2001) Chromogranin A, an "on/off" switch controlling dense-core secretory granule biogenesis. Cell 106:499–509

Kim T, Zhang CF, Sun Z, Wu H, Loh YP (2005) Chromogranin A deficiency in transgenic mice leads to aberrant chromaffin granule biogenesis. J Neurosci 25:6958–6961

Koshimizu H, Kim T, Cawley NX, Loh YP (2010) Chromogranin A: a new proposal for trafficking, processing and induction of granule biogenesis. Regul Pept 160:153–159

Kromer A, Glombik MM, Huttner WB, Gerdes HH (1998) Essential role of the disulfide-bonded loop of chromogranin B for sorting to secretory granules is revealed by expression of a deletion mutant in the absence of endogenous granin synthesis. J Cell Biol 140:1331–1346

Lee JC, Hook V (2009) Proteolytic fragments of chromogranins A and B represent major soluble components of chromaffin granules, illustrated by two-dimensional proteomics with NH(2)-terminal Edman peptide sequencing and MALDI-TOF MS. Biochemistry 48:5254–5262

Mahapatra NR, O'Connor DT, Vaingankar SM, Hikim AP, Mahata M, Ray S, Staite E, Wu H, Gu Y, Dalton N, Kennedy BP, Ziegler MG, Ross J, Mahata SK (2005) Hypertension from targeted ablation of chromogranin A can be rescued by the human ortholog. J Clin Invest 115:1942–1952

Mahata SK, Marksteiner J, Sperk G, Mahata M, Gruber B, Fischer-Colbrie R, Winkler H (1992) Temporal lobe epilepsy of the rat: differential expression of mRNAs of chromogranin B, secretogranin II, synaptin/synaptophysin and p65 in subfield of the hippocampus. Brain Res Mol Brain Res 16:1–12

Marksteiner J, Lechner T, Kaufmann WA, Gurka P, Humpel C, Nowakowski C, Maier H, Jellinger KA (2000) Distribution of chromogranin B-like immunoreactivity in the human hippocampus and its changes in Alzheimer's disease. Acta Neuropathol 100:205–212

Montero-Hadjadje M, Vaingankar S, Elias S, Tostivint H, Mahata SK, Anouar Y (2008) Chromogranins A and B and secretogranin II:

evolutionary and functional aspects. Acta Physiol (Oxf) 192:309–324

Montero-Hadjadje M, Elias S, Chevalier L, Benard M, Tanguy Y, Turquier V, Galas L, Yon L, Malagon MM, Driouich A, Gasman S, Anouar Y (2009) Chromogranin A promotes peptide hormone sorting to mobile granules in constitutively and regulated secreting cells: role of conserved N- and C-terminal peptides. J Biol Chem 284:12420–12431

Montesinos MS, Machado JD, Camacho M, Diaz J, Morales YG, Alvarez de la Rosa D, Carmona E, Castaneyra A, Viveros OH, O'Connor DT, Mahata SK, Borges R (2008) The crucial role of chromogranins in storage and exocytosis revealed using chromaffin cells from chromogranin A null mouse. J Neurosci 28:3350–3358

Munoz DG (1991) Chromogranin A-like immunoreactive neurites are major constituents of senile plaques. Lab Invest 64:826–832

Natori S, Huttner WB (1996) Chromogranin B (secretogranin I) promotes sorting to the regulated secretory pathway of processing intermediates derived from a peptide hormone precursor. Proc Natl Acad Sci USA 93:4431–4436

Nishimura M, Tomimoto H, Suenaga T, Nakamura S, Namba Y, Ikeda K, Akiguchi I, Kimura J (1994) Synaptophysin and chromogranin A immunoreactivities of Lewy bodies in Parkinson's disease brains. Brain Res 634:339–344

O'Connor DT, Burton D, Deftos LJ (1983) Immunoreactive human chromogranin A in diverse polypeptide hormone producing human tumors and normal endocrine tissues. J Clin Endocrinol Metab 57:1084–1086

Obermuller S, Calegari F, King A, Lindqvist A, Lundquist I, Salehi A, Francolini M, Rosa P, Rorsman P, Huttner WB, Barg S (2010) Defective secretion of islet hormones in chromogranin-B deficient mice. PLoS One 5:e8936

Park HY, So SH, Lee WB, You SH, Yoo SH (2002) Purification, pH-dependent conformational change, aggregation, and secretory granule membrane binding property of secretogranin II (chromogranin C). Biochemistry 41:1259–1266

Pihel K, Schroeder TJ, Wightman RM (1994) Rapid and selective cyclic voltammetric measurements of epinephrine and norepinephrine as a method to measure secretion from single bovine adrenal-medullary cells. Anal Chem 66:4532–4537

Schrott-Fischer A, Bitsche M, Humpel C, Walcher C, Maier H, Jellinger K, Rabl W, Glueckert R, Marksteiner J (2009) Chromogranin peptides in amyotrophic lateral sclerosis. Regul Pept 152:13–21

Sombers LA, Maxson MM, Ewing AG (2007) Multicore vesicles: hyperosmolarity and L-DOPA induce homotypic fusion of dense core vesicles. Cell Mol Neurobiol 27:681–685

Taupenot L, Harper KL, O'Connor DT (2003) The chromogranin–secretogranin family. N Engl J Med 348:1134–1149

Terland O, Flatmark T (1975) Ascorbate as a natural constituent of chromaffin granules from the bovine adrenal medulla. FEBS Lett 59:52–56

Trifaro JM, Glavinovic M, Rose SD (1997) Secretory vesicle pools and rate and kinetics of single vesicle exocytosis in neurosecretory cells. Neurochem Res 22:831–841

Videen JS, Mezger MS, Chang YM, O'Connor DT (1992) Calcium and catecholamine interactions with adrenal chromogranins. Comparison of driving forces in binding and aggregation. J Biol Chem 267:3066–3073

Weber A, Westhead EW, Winkler H (1983) Specificity and properties of the nucleotide carrier in chromaffin granules from bovine adrenal medulla. Biochem J 210:789–794

Wightman RM, Jankowski JA, Kennedy RT, Kawagoe KT, Schroeder TJ, Leszczyszyn DJ, Near JA, Diliberto EJ, Viveros OH (1991) Temporally resolved catecholamine spikes correspond to single vesicle release from individual chromaffin cells. Proc Nat Acad Sci USA 88:10754–10758

Winkler H, Westhead E (1980) The molecular organization of adrenal chromaffin granules. Neuroscience 5:1803–1823

Wolkersdorfer M, Laslop A, Lazure C, Fischer-Colbrie R, Winkler H (1996) Processing of chromogranins in chromaffin cell culture: effects of reserpine and alpha-methyl-*p*-tyrosine. Biochem J 316(Pt 3):953–958

Yoo SH (1996) pH- and Ca(2+)-dependent aggregation property of secretory vesicle matrix proteins and the potential role of chromogranins A and B in secretory vesicle biogenesis. J Biol Chem 271:1558–1565

Yoo SH (2010) Secretory granules in inositol 1,4,5-trisphosphate-dependent Ca^{2+} signaling in the cytoplasm of neuroendocrine cells. FASEB J 24(3):653–654

Yoo SH, Albanesi JP (1990) Ca2(+)-induced conformational change and aggregation of chromogranin A. J Biol Chem 265:14414–14421

Yoo SH, Albanesi JP (1991) High capacity, low affinity Ca^{2+} binding of chromogranin A. Relationship between the pH-induced conformational change and Ca^{2+} binding property. J Biol Chem 266:7740–7745

Zanner R, Gratzl M, Prinz C (2002) Circle of life of secretory vesicles in gastric enterochromaffin-like cells. Ann N Y Acad Sci 971:389–396

Zhang K, Rao F, Rana BK, Gayen JR, Callegary F, King A, Rosa P, Huttner WB, Stridsberg M, Mahata M, Vaingankar SM, Mahboubi V, Salem RM, Rodriguez-Flores JL, Fung MM, Smith DW, Schork NJ, Ziegler MG, Taupenot L, Mahata SK, O'Connor DT (2009) Autonomic function in hypertension: role of genetic variations at the catecholamine storage vesicle protein chromogranin B. Circ Cardiovasc Gen 2:46–56

Zhao E, Zhang D, Basak A, Trudeau VL (2009) New insights into granin-derived peptides: evolution and endocrine roles. Gen Comp Endocrinol 164:161–174