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REVIEW

Chromogranins as regulators of exocytosis

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Abstract

Chromogranins (Cgs) constitute the main protein component in the vesicular matrix of large dense core vesicles (LDCV). These acidic proteins have been implicated in several physiological processes such as vesicle sorting, the generation of bioactive peptides and the accumulation of soluble species inside LDCV. This latter feature of Cgs accounts for the ability of vesicles to concentrate catecholamines and Ca^{2+}. Indeed, the low affinity and high capacity of Cgs to bind solutes at the low pH of the LDCV lumen seems to be behind the delay in the neurotransmitter exit towards the extracellular milieu after vesicle fusion. The availability of new mouse strains lacking Cgs in combination with the arrival of several techniques for the direct monitoring of exocytosis (like amperometry, patch-amperometry and intracellular electrochemistry), have helped advance our understanding of how these granins concentrate catecholamines and Ca^{2+} in LDCV, and how they influence the kinetics of exocytosis. In this review, 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: adrenal, amperometry, chromaffin, secretogranin, secretion, secretory vesicle.

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An overview of chromogranins

A water-soluble glycoprotein that co-secretes with catecholamines from adrenomedullary cells was discovered in the mid-1960s (Banks and Helle 1965). This also provided the first direct evidence of exocytosis on the basis that these proteins could not be released in any other way. Indeed, a few years previously, an acidic protein was identified in large dense core vesicles (LDCV) of chromaffin cells (also called chromaffin granules:(Hillarp 1959) that was called chromogranin A (CgA) and that proved to be the first member of a family often denominated granins. The granins family of proteins has since expanded to also include chromogranin B (CgB or SgI), secretogranins II (SgII or CgC), SgIII (or 1B1075), SgIV (or HISL-19), SgV (or 7B2), SgVI (or NESP55), SgVII (or VGF) and non-acronymic, which are also found in many other tissues like the pituitary gland and islets of Langerhans, as well as in neurons in the brain (Taupenot *et al.* 2003; Helle 2009; Montero-Hadjadje *et al.* 2009; Zhao *et al.* 2009).

Several functions have been attributed to the Cgs. For instance, they have been implicated in the biogenesis of the LDCV, as well as the sorting mechanisms that occur in these vesicles (Glombik *et al.* 1999; Gerdes and Glombik 2000; Kim *et al.* 2001, 2005; Huh *et al.* 2003; 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; 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* because of 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). In addition, Cgs currently serve as tumor markers (O'Connor *et al.* 1983; Conlon 2009) 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) or neurodegenerative diseases like Parkinson's (Nishimura *et al.* 1994), Alzheimer's disease (Munoz 1991; Marksteiner

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Abbreviations used: CgA, chromogranin A; CgB, chromogranin B; KO, Knockout; LDCV, large dense core vesicles; Sg, secretogranin; V-ATPase, vesicular H^+-proton pump ATPase; WT, wild type.

et al. 2000), and amyotrophic lateral sclerosis (Schrott-Fischer *et al.* 2009).

However, the first function assigned to Cgs was in the concentration of catecholamines and other solutes inside the LDCV of secretory cells. Hence, the ability of Cgs to bind catecholamines is thought to help 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). Unexpectedly, these experiments showed that catecholamines from fused LDCV are released at a very slow rate (Wightman *et al.* 1991), evidence that a functional matrix retains these catecholamines. Indeed, 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 (Yoo 2009). Here we shall center our attention on the most relevant issues related to the participation of Cgs in amine accumulation in the LDCV and in their release, principally as studied in adrenal chromaffin cells.

Large dense core vesicles, a concentrated cocktail of solutes

The large variety of solutes present into chromaffin granules was defined as a vesicular cocktail (Winkler *et al.* 1998). A typical chromaffin cell contains 12 000–30 000 LDCV, although only a small fraction is suitable for immediate release (Heinemann *et al.* 1994; Trifaro *et al.* 1997). The adrenal-medullary response to stress requires the secretion of large amounts of catecholamines and peptides into the bloodstream, which is possible because of the surprisingly high concentration of solutes inside LDCV. For instance, catecholamines are estimated to be in the range of 0.5–1 M, although this figure might vary from one animal species to another (Winkler and Westhead 1980; Pihel *et al.* 1994; Albillos *et al.* 1997; Montesinos *et al.* 2008). In addition, vesicles 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 forms a condensed matrix together with Cgs at an acidic pH of ≈5.5 in the vesicles. The theoretical osmolarity of this mixture would be around 1500 mOsm. Hence, to maintain the homeostasis of LDCV and avoid their disruption because of osmotic stress, the functional aggregation of soluble species is necessary. 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 electron dense core. Indeed, the vesicular content of Cgs in bovine chromaffin LDCV is estimated to be ≈1800 μM of CgA, ≈200 μM of CgB and ≈30 μM of SgII (Yoo 2009), although their relative amounts may also vary depending on the species.

Chromogranins are currently considered as a saturable high capacity and low affinity sink. For instance, CgA binds 32 mol adrenaline per mol with a K_d of 2.1 mM (Videen *et al.* 1992), and Cgs also bind Ca^{2+} ≈50 mol per mol of Cg with a K_d of 1.5–4 mM depending on the type of granin (Yoo and Albanesi 1991; Yoo 2009). The ability of CgA and CgB to interact with each other to form dimers or hetero-tetramers has been studied in order to explore the interaction of Cgs with Ca^{2+} (Yoo and Albanesi 1991; Yoo 1996). However, the same interaction with soluble species like 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).

We can divide intravesicular compounds 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' as they are moving in and out of the vesicle, whereas Cgs and other proteins like enzymes are '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 principle candidates to be involved in the regulation of exocytosis as changes in any one of these species will affect the others.

In addition to the naturally occurring compounds, several drugs have been recognized as false neurotransmitters like α-methy-norepinephrine or tyramine, and they can accumulate in secretory vesicles displacing the natural species (Crout *et al.* 1962; Philippu and Schumann 1965). Other weak bases like amphetamines have received attention as they were seen to accumulate in a pH-dependent manner inside vesicles, reducing the quantum size (the amount of catecholamines released per single exocytotic event) by displacing catecholamines towards the cytosol (Sulzer *et al.* 1993; Fon *et al.* 1997; Mundorf *et al.* 1999). However, many other drugs share this characteristic of being permeable weak bases, a circumstance that is frequently ignored that they accumulated strongly in the acidic organelles like secretory vesicles, and that many of them also bind to Cgs. We described how anti-hypertensive drugs like hydralazine (Machado *et al.* 2002b) or β-adrenergic blockers (Montesinos *et al.* 2010) accumulate in the LDCV and reduce the quantum size of catecholamine exocytosis. The accumulation of such drugs inside the vesicles could also produce drastic changes in the composition of the 'mobile components', like amines, Ca^{2+} and perhaps ATP.

The acidic nature of the LDCV is a crucial factor in understanding the equilibrium of its inner components.

Hence, the pH of vesicles (5.5) coincides with the maximum stability of Cgs and their optimal capacity to bind soluble species (Helle *et al.* 1985). Their high content of glutamic and aspartic acid residues produces a pI of 4.4–5.4 (Falkensammer *et al.* 1985). The H^+ gradient across the LDCV membrane is also responsible for maintaining the high concentrations of amines, Ca^{2+} and ATP inside the vesicles as it is the counter-ion used by the carriers of these species (Henry *et al.* 1998). The pH gradient depends on the activity of a vesicular H^+-proton pump ATPase (V-ATPase), which is continuously pumping H^+ to acidify the vesicle (Nelson and Harvey 1999). The role of the pH gradient can be tested by following the effects of rapid vesicle alkalinization, for instance using the V-ATPase blocker bafilomycin. Bafilomycin reduces the quantum size and slows down the catecholamine release by exocytosis, as readily observed by amperometry (Camacho *et al.* 2006).

The regulation of the pH gradient across the LDCV membrane is probably the target of several second messengers which modify the kinetics of exocytosis, and our group has explored two of these second messenger signaling pathways (Borges *et al.* 2002). For example, the activation of the classical cyclic guanosine monophosphate/protein kinase G pathway by nitric oxide and other agents promotes the slowing down of catecholamine release in single exocytotic events, without changing the quantum size, an effect that can be rapidly reverted using nitric oxide scavengers (Machado *et al.* 2000). Similar results were found after activation of the cyclic adenosine monophosphate/protein kinase A pathway, although strong stimulation of this kinase also causes a notable increase in quantum size (Machado *et al.* 2001; Borges *et al.* 2002). Other drugs like estrogens also slow down exocytosis through a non-genomic mechanism that involves cAMP (Machado *et al.* 2002a). The activation of these two pathways produces a rapid alkalinization of LDCV (Camacho *et al.* 2006).

Vesicles behave like a bi-compartmental storage site where the free portion accounts for only ≈10% of the total catecholamines (Schroeder *et al.* 1996), this portion is probably associated with the halo observed in electron microscopy (Colliver *et al.* 2000). pH changes will rapidly affect this free fraction that will initially change the kinetics of exocytosis without altering the quantum size. However, strong or long lasting inhibition of the V-ATPase also causes the leakage of amines and other soluble components like Ca^{2+} and ATP, which despite the decrease in the quantum content also promotes granule movement and exocytosis (Camacho *et al.* 2006, 2008).

The delayed release of catecholamines during the exocytosis of LDCV

The release of adrenaline following single LDCV fusion events occurs two-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. One might be the diameter of the fusion pore that could limit the free escape of soluble species from the vesicle. The second candidate is the slow diffusion of solutes from the LDCV matrix (Schroeder *et al.* 1996; Amatore *et al.* 2000). 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, secretory vesicles from chromaffin and mast cells behave identically to changes in temperature and ionic composition in spite of their different matrix composition (Pihel *et al.* 1996). It is likely that the chromaffin matrix of LDCV swells and shrinks as was described in matrix from mast cells in beige mice (Marszalek *et al.* 1995). Exocytosis is also 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. Electron microscopy shows many small vesicles that contain little dense material and by amperometry, exocytosis was observed as secretory spikes that were drastically accelerated (Ardiles *et al.* 2006), suggesting a close relationship between the presence of vesicular matrix and the kinetics of exocytosis.

Catecholamine exocytosis in the absence of chromogranin A

The targeted ablation of the CgA gene in mice to generate CgA-knockout (KO) animals that were viable and fertile in homozygosis (Mahapatra *et al.* 2005; Hendy *et al.* 2006) 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 considered with care because of the partial compensation by the overexpression of CgB (Fig. 1a and b), the redistribution of Cgs produced drastic effects in the storage and release of catecholamines from the LDCV of adrenal chromaffin cells.

Despite the proposed role of CgA in granule biogenesis and sorting, chromaffin cells from CgA-KO can still release adrenaline by exocytosis. Using amperometry as the method of quantification (Fig. 1c and d) we concluded that cells from CgA-KO cell released ≈40% less catecholamines after a depolarizing stimulus (Fig. 1d), and that the number of

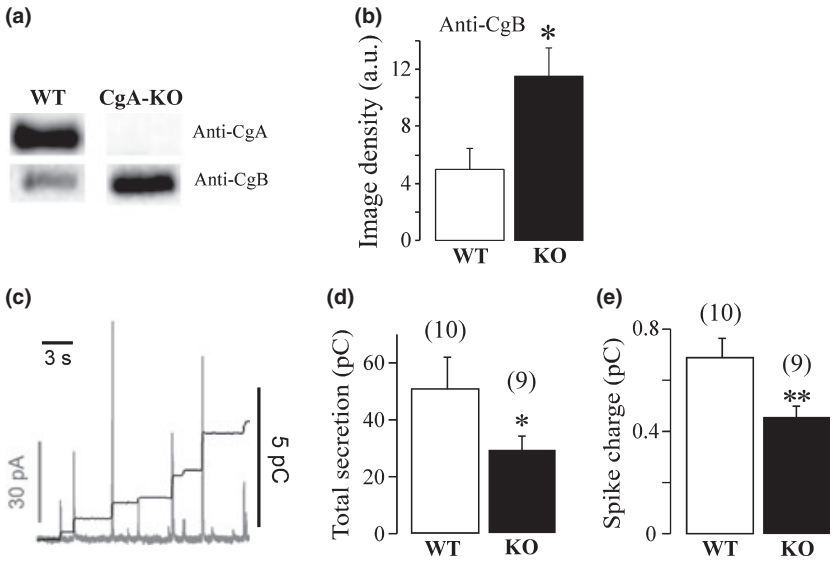

Fig. 1 Secretory characteristics of the CgA-KO mouse. (a) Western blots of the adrenal medulla confirming the lack in CgA and the over-expression of CgB. (b) Quantification of CgB in the WT and CgA-KO as the average from three wells containing four different medullas (means ± SEM). (c) Fragment from a typical amperometric trace from a KO chromaffin cell (units expressed in pAmperes, grey trace) with the cumulative secretion obtained by integration of the original trace superimposed (expressed in pCoulombs, black trace). (d) Analysis of the cumulative secretion over a 2-min recording (means ± SEM). (e) The net spike charge (Q, expressed in pC) from WT and KO animals. The number of cells in each condition is expressed in brackets. *$p < 0.05$; **$p < 0.01$ Mann–Whitney test. Modified from (Montesinos *et al.* 2008).

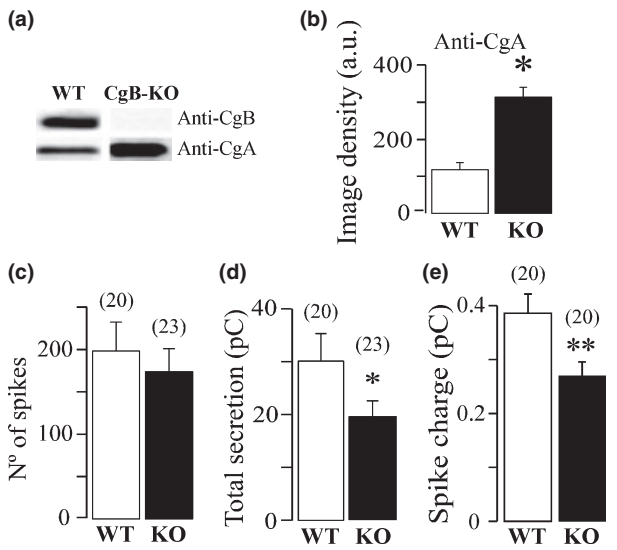

Fig. 2 Secretory characteristic of the CgB-KO mouse. (a) Western blot showing the absence of CgB and the over-expression of CgA. (b) Quantification of the CgA expression in 12 medullas from WT and CgB-KO animals. The gel density is expressed in arbitrary units. (c) Temporal distribution of secretory spikes over a 2-min recording. (d) Cumulative secretion obtained as described in the legend of Fig. 1. (e) The net spike charge (Q, expressed in pCoulombs) from WT and KO animals. *$p < 0.05$; **$p < 0.01$ Mann–Whitney test.

spikes from the wild type (WT) and CgA-KO cells was similar over a 2-min recording (126 ± 25 CgA-KO vs. 117 ± 18 WT). In addition, the net content of catecholamines per quantum (Q) was reduced by 34% and the kinetic analysis of secretory spikes showed that exocytosis occurred faster in CgA-KO cells, these kinetic changes mainly affecting the last part of spikes (Fig. 3). Taken together these data indicate that the matrix of LDCV without CgA is less capable of concentrating and retaining catecholamines, causing exocytosis to occur faster (Montesinos *et al.* 2008).

A second important consequence of the lack in CgA was revealed from experiments in which cells were overloaded with the catecholamine precursor L-DOPA. L-DOPA penetrates the chromaffin cell membranes and it is rapidly converted into dopamine, which is usually taken up by LDCV where the dopamine is converted to 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; Gong *et al.* 2003; Sombers *et al.* 2007) as observed in cells from WT mice. However, the LDCV of chromaffin cells from CgA-KO could not take up more amines.

To check whether this impediment to store more catecholamines was derived from a reduction in the availability of cytosolic catecholamines, we performed experiments using intracellular electrochemistry in the presence of the monoamino oxidase inhibitor pargyline. 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 (Mosharov *et al.* 2003). Chromaffin cells from KO animals had less free catecholamines than their WT counterparts. However, there

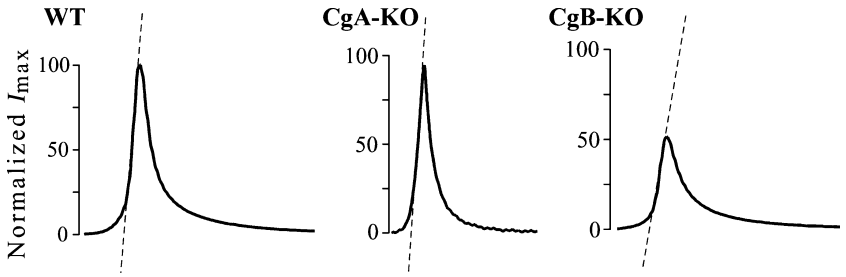

Fig. 3 Kinetic profiles of amperometric spikes from CgA- and CgB-KO chromaffin cells. Traces illustrate the kinetic changes observed in exocytosis from the cells lacking in CgA or CgB. The spikes were constructed by averaging spikes from WT, CgA-KO and CgB-KO and normalized to the I_{max} (100%) of their 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. Modified from (Diaz-Vera *et al.* 2010).

was a drastic 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).

The storage and release properties of LDCV lacking CgA were studied in more detail using patch-amperometry in the cell-attached configuration to monitor simultaneously the vesicle size (capacitance) and the release of catecholamines from the same vesicle (amperometry). These data revealed that the vesicular concentration of catecholamines drops from 870 mM in WT to 530 mM in the CgA-KO.

Taking these data together, we conclude that the LDCV from chromaffin cells lacking CgA have a dramatically weaker capacity to accumulate catecholamines, even considering the over-expression of CgB.

Catecholamine exocytosis in the absence of chromogranin B

The first mouse strain lacking CgB was obtained in the laboratory of Wieland Huttner in Heidelberg (Germany). Although indistinguishable from the wildtypes at first sight, these mice developed a phenotype of hypertension (Zhang *et al.* 2009) and intolerance to glucose (Obermuller *et al.* 2010). In contrast to the CgA-KO mouse, immunohistochemistry and western blotting confirmed the absence of CgB and the over-expression of CgA (Fig. 2a and b). We carried out experiments to examine the secretory characteristics of chromaffin cells in these mice in a similar way to that followed for the CgA-KO strain.

Chromogranin B has been proposed as a critical factor in the genesis and sorting of LDCV (Natori and Huttner 1996; Kromer *et al.* 1998; Glombik *et al.* 1999). 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. 2c). However, the total amount of catecholamines released was reduced by 33% (Fig. 2d), roughly coinciding with the amount released per quanta (Fig. 2e). 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).

Using the same experimental approach as that employed with CgA-KO cells, 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 over-expressed in the adrenomedullary tissues of the CgB-KO mice. Moreover, the cells were competent to release catecholamines with a similar frequency of events as WT cells, although exocytosis exhibited a drastic 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.

To determine whether other granins, in addition to CgA, could fulfill the role of CgB in forming the dense matrix, we performed a proteomic analysis of the enriched LDCV fraction from mouse adrenal medullas. This kind of study on the LDCV of mouse chromaffin cells is not easy to perform because of 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. The major differences in the proteins expressed by the WT (C57BL/6J) and the CgB-KO adrenomedullary LDCV are shown in Table 1. 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

Table 1 Proteins present in LDCV of CgB-KO chromaffin cells identified by MALDI-TOF MS

Protein identity	NCBI No (gi)	MW (Da)	pI	Fold change
Chromogranin-B	50409	77 895	5.01	Only WT
Secretogranin II, precursor	417771	70 600	4.69	–[a]
Chromogranin-A	20071660	51 929	4.65	–[a]
Aldehyde dehydrogenase 2, mitochondrial	26330458	57 015	7.53	+2.4
Albumin	26341396	67 013	5.49	−2.3
Heat shock protein 1 (Chaperonin)	26353954	61 089	5.67	+1.8
Protein disulfide isomerase associated 3	26353794	57 103	5.78	+1.5
Vimentin	2078001	51 590	4.96	Only KO
Fibrinogen, gamma polypeptide	18043449	50 044	5.54	Only KO
Fibrinogen, beta polypeptide	21619364	55 402	6.68	Only KO
Fibrinogen, alpha polypeptide	13529485	61 801	7.16	Only KO
ATP synthase, H^+ transporting mitochondrial F1 complex, beta subunit	28302366	56 265	5.19	+1.3
Acyl-Coenzyme A dehydrogenase, short chain	192659	45 203	8.96	Only KO
Apolipoprotein E	54035417	35 901	5.56	+2.6
Prohibitin	12832901	29 859	5.57	+1.5
Chapsin 110/Discs large homolog 2 (Drosophila)	26338173	54 421	6.93	−2.1

[a]Not quantified, multiple post-processing proteins.

of other proteins (Table 1) and when we combed the areas of the 2D gels where other granins should be present, none of them were over-expressed. 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 in the release of bioactive peptides originating from CgB and/or from the over-expressed CgA (Obermuller *et al.* 2010).

The marked reduction of CgB in brain neuron terminals (Marksteiner *et al.* 2000) and polymorphisms in apolipoprotein E have been associated with Alzheimer's disease, and together with the reduction observed in the expression of chapsin 110, these animals lacking CgB might represent a potentially interesting model of neurodegenerative diseases. Chaperonin and the disulphide isomerase 3 associated protein are involved in the folding that occurs in the endoplasmic reticulum and they could be packaged into LDCV (Table 1). Only those proteins which show changes in their expression compared to WT were quantified. This excluded a number of well-established vesicular proteins like dopamine-β-hydroxylase. As the amount of starting material was so small, it is also likely that certain proteins were undetectable (Apps 1997).

Surprisingly, the study (Diaz-Vera *et al.* 2010) 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 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).

Concluding remarks

New data obtained from Cgs-KO mice have provided direct evidence implicating Cgs in vesicular storage and in the exocytotic release of catecholamines. It have been 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 catecholamines in Cgs-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 over-expression 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 CgB-KO animals. In conclusion, Cgs are highly efficient systems directly involved in monoamine accumulation and in the kinetics of exocytosis from LDCV.

Since their discovery, Cgs have captivated the attention of scientists, expanding the functions initially proposed to a wide range of roles that include granule biogenesis and sorting, 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 exocytosis

from the LDCV. The generation of mice strains lacking either in CgA and CgB has opened the possibility to study 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 drastically. New data have highlighted the role of Cgs in the maintenance of the 'mobile components' inside LDCV (Yoo 2010), where each protein CgA and CgB exhibited clear differences in their ability to bind and release neurotransmitters (Montesinos *et al.* 2008; Diaz-Vera *et al.* 2010; Yoo 2010). We are currently studying exocytosis in the absence of both Cgs using a new mouse strain lacking both CgA & B.

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