DE GRUYTER DOI 10.1515/bmc-2013-0020 — BioMol Concepts 2013; 4(6): 605–609

Short Conceptual Overview

Judith Estevez-Herrera, Marta R. Pardo, Natalia Dominguez, Daniel Pereda, Jose D. Machado and Ricardo Borges*

The role of chromogranins in the secretory pathway

Abstract: Chromogranins (Cgs) are acidic proteins implicated in several physiological processes, including the biogenesis and sorting of secretory vesicles, the generation of bioactive peptides, and the accumulation of soluble species inside large dense core vesicles (LDCV). Indeed, Cgs are the main protein component of the vesicular matrix in LDCV, and they are involved in the concentration of soluble species like neurotransmitters and calcium. Experiments using electrochemical techniques such amperometry, patch amperometry, and intracellular electrochemistry have clarified the functional roles of Cgs in the accumulation and release of catecholamines. We have focused this review at a single event of exocytosis of chromaffin cells from three mouse strains lacking Cgs. Accordingly, in this brief review, we will focus on the role of Cgs in maintaining the intravesicular environment of secretory vesicles and in exocytosis, bringing together the most recent findings from studies on adrenal chromaffin cells.

Keywords: adrenal; chromaffin; exocytosis; granins; secretogranin II.

***Corresponding author: Ricardo Borges,** Unidad de Farmacología, Facultad de Medicina, Universidad de La Laguna, E-38071 La Laguna, Tenerife, Spain; and Instituto de BioOrgánica 'Antonio González', Universidad de La Laguna, E-38071 La Laguna, Tenerife, Spain, e-mail: rborges@ull.es
Judith Estevez-Herrera, Natalia Dominguez, Daniel Pereda and Jose D. Machado: Unidad de Farmacología, Facultad de Medicina, Universidad de La Laguna, E-38071 La Laguna, Tenerife, Spain
Marta R. Pardo: Unidad de Farmacología, Facultad de Medicina, Universidad de La Laguna, E-38071 La Laguna, Tenerife, Spain; and Instituto de BioOrgánica 'Antonio González', Universidad de La Laguna, E-38071 La Laguna, Tenerife, Spain

List of abreviations

CgA, chromogranin A; CgB, chromogranin B; Cgs, chromogranins; LDCV, large dense core vesicles.

Introduction

In the mid-sixties, chromogranin A was discovered in the large dense core vesicles (LDCV) of chromaffin cells (also called chromaffin granules) (1), and it proved to be the first member of the subsequently identified granin family. A number of granins have since been described including 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 SgVIII (or proSAAS). These other members of the family are found in a variety of tissues, such as the pituitary gland and islets of Langerhans, as well as in neurons (2).

The chromogranins have been implicated in the biogenesis of LDCV and in their sorting mechanisms (3–5). In addition, chromogranins (Cgs) are prohormones that constitute a source of bioactive peptides [reviewed in (2, 6, 7)] and recently, Cgs were proposed to be directly involved in the development of some neurological diseases like schizophrenia, epilepsy, or neurodegenerative diseases, including Parkinson's, Alzheimer's disease, and amyotrophic lateral sclerosis (8).

Chromogranins as key intravesicular proteins for the aggregation of soluble species

The first function assigned to Cgs involved their contribution to the concentration of catecholamines and other solutes within the LDCV of secretory cells. The ability of Cgs to bind catecholamines is thought to help reduce osmotic pressure and prevent the swelling vesicles from bursting (9). This hypothesis regarding the adsorption of catecholamines to Cgs received strong support when amperometric recordings became available. Amperometry showed that the release of catecholamines during single secretory events occurs very slowly, suggesting that a functional matrix retains these solutes.

A typical chromaffin cell contains 12,000 to 30,000 LDCV, although only a small fraction of these are suitable for immediate release. The adrenal-medullary response to stress involves the secretion of large amounts of catecholamines and peptides into the bloodstream. The intravesicular cocktail (10) contains surprisingly high concentrations of catecholamines, close to the molar range, and in addition, vesicles contain large amounts of ATP, Ca^{2+}, ascorbate, as well as ill-defined amounts of biopeptides, other nucleotides, dopamine-β-hydroxylase, and H^{+}. All of these components, together with Cgs, seem to form the condensed vesicle matrix that establishes an inner acidic pH of ≈5.5, and the theoretical osmolarity of this mixture is around 1500 mOsm. Hence, to maintain the homeostasis of LDCV and avoid their disruption due to osmotic stress, it is necessary to functionally aggregate soluble species. As Cgs 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 that is evident by electron microscopy. Indeed, the vesicular content of Cgs in bovine chromaffin LDCV is estimated to be ≈1800 μM of chromogranin A (CgA), ≈200 μM of CgB, and ≈30 μM of SgII (11), although their relative amounts vary depending on the species.

One mole of CgA is thought to bind 32 mol of adrenaline, with a Kd of 2.1 mM (12), and Cgs can also bind Ca^{2+} ≈50 mol/mol of Cg, with a Kd of 1.5 to 4 mM depending on the type of granin. All mobile compounds of the vesicular cocktail (amines, ascorbate, H^{+}, Ca^{2+}, and ATP) are in asymmetric equilibrium with the cytosol and the matrix, and as such, the strength of this association is a candidate for regulating the kinetics of exocytosis. It should also be taken into account that several drugs like α-methylnorepinephrine and tyramine, or weak bases like amphetamines (13), hydralazine (14), antipsychotic drugs, or β-adrenergic blockers (15), accumulate in a pH-dependent manner in LDCV, reducing the quantum size by displacing catecholamines. Although frequently ignored, the sequestering of drugs into acidic secretory organelles could produce drastic changes in their composition.

The optimal capacity of Cgs to bind soluble species is at the pH of the vesicles (≈5.5) (9). Maintaining this pH gradient depends on the activity of a vesicular ATPase H^{+}-proton pump (V-ATPase), which continuously pumps H^{+} to acidify the vesicle. This H^{+} gradient across the vesicle membrane provides counterions for the carriers of amines, Ca^{2+}, and ATP. A crucial tool to study the pH gradient is the V-ATPase blocker bafilomycin, which reduces the quantum size of vesicles and slows down catecholamine release by exocytosis, as readily observed by amperometry (16).

Several second messenger cascades affect the kinetics of exocytosis, probably by reducing the pH gradient across the LDCV membrane (16). This is the case of the activation by NO of the cGMP/PKG pathway, which slows down catecholamine release through single exocytosis (17). Similar results were found after activation of the cAMP/PKA pathway, although strong stimulation of this kinase also causes a notable increase in quantum size (18, 19). Other drugs like estrogens also slow down exocytosis through a nongenomic mechanism that involves cAMP (20).

The release of adrenaline following single LDCV fusion events occurs at least two to three orders of magnitude slower than that predicted by the diffusion coefficient of catecholamines in aqueous media (21, 22). The retention of catecholamines inside the fused vesicle could be due to distinct properties: the diameter of the fusion pore, the slow diffusion of solutes from the LDCV matrix (23), and/or the presence of extracellular polysaccharides. Patch amperometry, which combines amperometry with cell-attached capacitance measurements, revealed that the arrival of catecholamines to the carbon fiber electrode was delayed even when the fusion pore is supposedly dilated (24). This would suggest the direct involvement of the vesicle matrix in the slow release of amines observed once vesicle fusion has taken place.

Secretory vesicles from chromaffin and mast cells respond identically to changes in temperature and ionic composition, despite their distinct compositions (25). Indeed, it is likely that the chromaffin matrix of LDCV swells and shrinks in a similar fashion to the matrix from mast cells in beige mice (26). Exocytosis is also largely delayed in the presence of cross-linking agents like glutaraldehyde or formaldehyde, which, after entering through the fusion pore during exocytosis, would freeze the dissociation of catecholamines from Cgs (27). By contrast, the opposite was observed in chromaffin cells cultured in astrocyte-conditioned media, where exocytosis is drastically accelerated (28), suggesting a close relationship between the presence of vesicular matrix and the kinetics of exocytosis.

Exocytosis in mice lacking chromogranins

We have studied the kinetics of exocytosis and the cargo capacity of LDCV from mice lacking CgA, CgB, or both. CgA-KO mice, which were reported to be viable and fertile in homozygosis (29), provided the first opportunity to study exocytosis in the absence of one of the 'immobile components' of chromaffin vesicles. This alteration produces drastic effects in the LDCV of adrenal chromaffin cells, and the lack of CgA was associated with the overexpression of CgB (Figure 1A and B). Despite the proposed role of CgA in granule biogenesis and sorting, chromaffin cells from CgA-KO still produce functional secretory granules that release adrenaline by exocytosis. Using amperometry, we found that depolarizing stimuli provoke the release of ≈40% less catecholamines in CgA-KO cells (Figures 1C and D). However, the number of spikes from the WT and CgA-KO cells was similar, even though the net content of catecholamines per quantum (Q) was reduced by 34%. Indeed, kinetic analysis of secretory spikes showed that exocytosis occurred faster in CgA-KO cells, in which such kinetic changes mainly affect the later part of the spikes [Figure 2, data from (24)].

The catecholamine precursor L-DOPA penetrates chromaffin cell membranes, and it is rapidly converted into dopamine, which is in turn transformed into noradrenaline by dopamine-β-hydroxylase. In WT mice, L-DOPA increases the vesicular content of catecholamines (30), although the LDCV from CgA-KO chromaffin cells cannot take up more amines. If catecholamines cannot be taken up by LDCV,

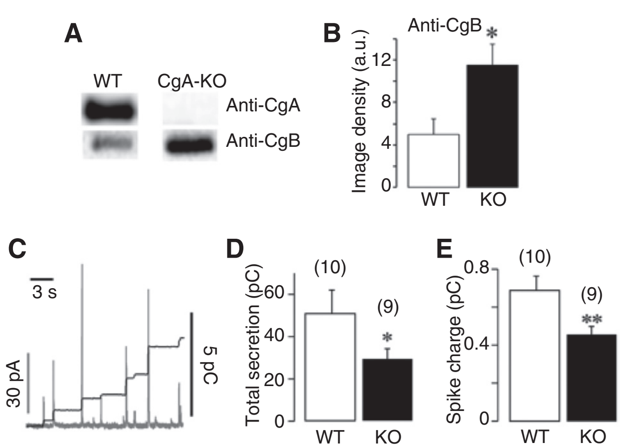

Figure 1 Secretory characteristics of the CgA-KO mouse.
(A) Western blot of the adrenal medulla confirming the lack of CgA and the overexpression of CgB. (B) Quantification of CgB in the WT and CgA-KO as the average from six different analyses (means±SEM). (C) Fragment from a typical amperometric trace from a KO chromaffin cell (units expressed in pAmperes – gray trace) with the cumulative secretion evident by integrating the original superimposed trace (expressed in pCoulombs – black trace). (D) Analysis of cumulative secretion over a 2-min recording (mean±SEM). (E) The net spike charge from WT and KO animals (Q, expressed in pC). The number of cells in each condition is shown in brackets: *$p<0.05$; **$p<0.01$ Mann-Whitney test. Modified from (24).

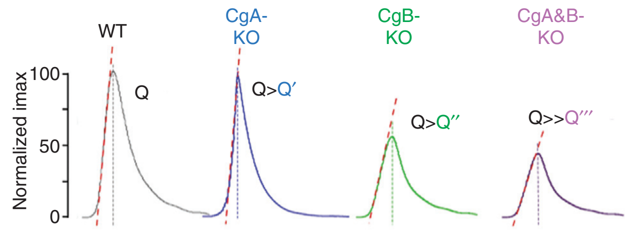

Figure 2 Kinetic profiles of amperometric spikes from Cg-KO chromaffin cells.
The traces illustrate the kinetic changes observed in exocytosis from cells lacking Cgs. The spikes were constructed by averaging the spikes from WT, CgA-, CgB-, and CgA/B-KO chromaffin cells, and then normalizing them to the Imax (100%) of their own control cells (C57BL/6). 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 (34).

they should remain present in the cytosol. Intracellular electrochemistry can provide access to the cytosol in order to measure cytosolic amines. Cell incubation with L-DOPA produces a large increase in cytosolic catecholamines in CgA-KO chromaffin cells compared to WT cells. Hence, the newly synthesized amines appear to be unable to enter the LDCV as the saturated matrix leaves no room for new catecholamines. Using patch amperometry, we found that the vesicular concentration of catecholamines drops from 870 mM in WT to 530 mM in the CgA-KO (24).

The effect of vesicular CgB has been analyzed in chromaffin cells from CgB-KO mice (31), and as in the CgA-KO, this mouse overexpressed the complementary CgA (Figures 3A and B). Amperometry recordings show that chromaffin cells from WT and CgB-KO mice exhibited similar secretory patterns, with no differences in the number of spikes (Figure 3C). However, the total amount of catecholamines released was again reduced by 33% (Figure 3D), roughly coinciding with the amount released per quanta (Figure 3E). A careful analysis of the kinetic properties of secretory spikes revealed a slowing of exocytosis that, by contrast to that observed in the CgA-KO mouse, largely affected the first (ascending) part of the spikes (32) (Figure 2).

As what occurs in CgA-KO cells, L-DOPA overloading demonstrated that LDCV from CgB-KO mice cannot take up more catecholamines, and amines that are unable to enter the vesicles remain in the cytosol. However, cells are still competent to maintain a similar frequency of secretory events than WT cells, even though they are associated with a drastic reduction in quantum size of individual events. This latter reduction produced an important fall in the total secretory response of chromaffin cells. In addition, the kinetics of exocytosis is affected during the initial

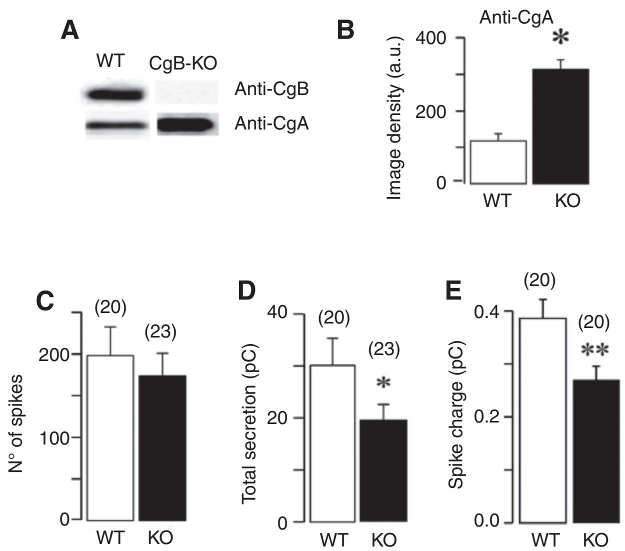

Figure 3 Secretory characteristics of the CgB-KO mouse. (A) Western blot showing the absence of CgB and the overexpression of CgA. (B) Quantification of CgA expression in WT and CgB-KO animals from three different experiments, the densitometry analysis is expressed in arbitrary units. (C) Temporal distribution of secretory spikes over a 2-min recording. (D) Cumulative secretion obtained as described in the legend to Figure 1. (E) The net spike charge (Q, expressed in pCoulombs) from WT and KO animals: **p*<0.05; ***p*<0.01 Mann-Whitney test. Modified from (32).

part of the spikes (Figure 2), and the LDCV from CgB-KO cells have their storage mechanisms saturated.

Given that we have CgA-KO and CgB-KO mice, we generated the double CgA/B-KO mouse that was both viable and fertile in homozygosis (33). With a few exceptions, what we have observed in this double Cg-KO mouse is an exacerbation of the phenotype observed with the individual Cg-KOs (Figure 2). In other words, the quantum size was halved, the ability of granules to take up amines was impaired, and the cytosolic amines that accumulated in response to L-DOPA exposure rose. By contrast to the CgA- or CgB-KO, the frequency of exocytosis was also reduced in the double CgA/B-KO, affecting the total catecholamines released (34).

Electron microscopy revealed the presence of giant and highly altered secretory vesicles with a poor electron-dense inner matrix. However, in the absence of Cgs, which proteins are responsible for the generation of even this impoverished electron-dense matrix? Proteomic analysis of the enriched LDCV fraction from the adrenal medulla of the CgA/B-KO mouse (33) shows no significant changes in the amount of SgII or other Cgs. Yet, surprisingly, significant amounts of fibrinogen are detected, of which the three chains (α, β, and γ) are only present in the LDCV of the CgB- and CgA/B-KO mice. In addition to its crucial role in clot formation, fibrinogen has been associated with the sorting of constitutive vesicles, and indeed, no other protein appears to be capable of fulfilling the functional role of Cgs as a matrix condenser for soluble intravesicular components. Nevertheless, even in the total absence of Cgs, the quantum catecholamine content is halved, and the remaining amines are still above isotonic concentrations in the cytosol. As such, we cannot rule out the possibility that other components of the vesicular cocktail, such as ATP (35) or Ca^{2+}, could be contributing to the maintenance of isotonicity and permitting amine accumulation.

Since the first description of Cgs, new functions have been proposed from those initially assigned. In this review, we have addressed some functional features of the roles of Cgs in exocytosis from LDCV. The generation of mice strains lacking Cgs has opened the possibility to study the role of such ‘immobile components.’ New data have highlighted the role of Cgs in maintaining ‘mobile components’ inside the LDCV (11), where CgA and CgB proteins exhibited clear differences in their ability to bind and release neurotransmitters (11, 24, 32).

Our current view about the role of Cgs in the secretory process can be resumed as follows: (i) Secretory events persist even in the complete absence of Cgs indicating that granins are not necessary for the biogenesis or for the maintenance of the secretory function. (ii) The lack of Cgs impairs the vesicular accumulation of CA, suggesting that the capacity of vesicles to store catecholamines is saturated. (iii) The protein analysis of the secretory vesicle fraction showed the compensatory overexpression of one Cg when the other is absent. (iv) Other proteins apparently unrelated to the secretory process were present in the vesicular fraction of CgA/B-KO animals.

Acknowledgments: J.E.-H. is recipient of an FPI (Spanish Ministry of Science and Innovation (MICINN). J.D.M. is funded by a Ramon y Cajal contract (R&C-2010-06256, MICINN/ERDF). This work was supported by a MICINN Grant BFU2010-15822, CONSOLIDER (CSD2008-00005) to R.B.

Received July 5, 2013; accepted September 20, 2013

References

1. Banks P, Helle K. The release of protein from the stimulated adrenal medulla. Biochem J 1965; 97: 40C–1C.
2. Taupenot L, Harper KL, O'Connor DT. The chromogranin-secretogranin family. N Engl J Med 2003; 348: 1134–49.
3. Kim T, Tao-Cheng JH, Eiden LE, Loh YP. Chromogranin A, an 'on/off' switch controlling dense-core secretory granule biogenesis. Cell 2001; 106: 499–509.
4. Huh YH, Jeon SH, Yoo SH. Chromogranin B-induced secretory granule biogenesis: comparison with the similar role of chromogranin A. J Biol Chem 2003; 278: 40581–9.
5. Gerdes HH, Glombik MM. Signal-mediated sorting of chromogranins to secretory granules. Adv Exp Med Biol 2000; 482: 41–54.
6. Montero-Hadjadje M, Vaingankar S, Elias S, Tostivint H, Mahata SK, Anouar Y. Chromogranins A and B and secretogranin II: evolutionary and functional aspects. Acta Physiol (Oxf) 2008; 192: 309–24.
7. Zhao E, Zhang D, Basak A, Trudeau VL. New insights into granin-derived peptides: evolution and endocrine roles. Gen Comp Endocr 2009, 164: 161–74.
8. Bartolomucci A, Possenti R, Mahata SK, Fischer-Colbrie R, Loh YP, Salton SR. The extended granin family: structure, function, and biomedical implications. Endocr Rev 2011; 32: 755–97.
9. Helle KB, Reed RK, Pihl KE, Serck-Hanssen G. Osmotic properties of the chromogranins and relation to osmotic pressure in catecholamine storage granules. Acta Physiol Scand 1985; 123: 21–33.
10. Winkler H, Westhead E. The molecular organization of adrenal chromaffin granules. Neuroscience 1980; 5: 1803–23.
11. Yoo SH. Secretory granules in inositol 1,4,5-trisphosphate-dependent Ca2+ signaling in the cytoplasm of neuroendocrine cells. FASEB J 2010; 24: 653–64.
12. Videen JS, Mezger MS, Chang YM, O'Connor DT. Calcium and catecholamine interactions with adrenal chromogranins. Comparison of driving forces in binding and aggregation. J Biol Chem 1992; 267: 3066–73.
13. Sulzer D, Maidment NT, Rayport S. Amphetamine and other weak bases act to promote reverse transport of dopamine in ventral midbrain neurons. J Neurochem 1993; 60: 527–35.
14. Machado JD, Gomez JF, Betancor G, Camacho M, Brioso MA, Borges R. Hydralazine reduces the quantal size of secretory events by displacement of catecholamines from adrenomedullary chromaffin secretory vesicles. Circ Res 2002; 91: 830–6.
15. Montesinos MS, Camacho M, Machado JD, Viveros OH, Beltran B, Borges R. The quantal secretion of catecholamines is impaired by the accumulation of beta-adrenoceptor antagonists into chromaffin cell vesicles. Br J Pharmacol 2010; 159: 1548–56.
16. Camacho M, Machado JD, Montesinos MS, Criado M, Borges R. Intragranular pH rapidly modulates exocytosis in adrenal chromaffin cells. J Neurochem 2006; 96: 324–34.
17. Machado JD, Segura F, Brioso MA, Borges R. Nitric oxide modulates a late step of exocytosis. J Biol Chem 2000; 275: 20274–9.
18. Machado JD, Morales A, Gomez JF, Borges R. cAMP modulates exocytotic kinetics and increases quantal size in chromaffin cells. Mol Pharmacol 2001; 60: 514–20.
19. Borges R, Machado JD, Betancor G, Camacho M. Pharmacological regulation of the late steps of exocytosis. Ann N Y Acad Sci 2002; 971: 184–92.
20. Machado JD, Alonso C, Morales A, Gomez JF, Borges R. Nongenomic regulation of the kinetics of exocytosis by estrogens. J Pharmacol Exper Ther 2002; 301: 631–7.
21. Gerhardt G, Adams RN. Determination of diffusion-coefficients by flow-injection analysis. Anal Chem 1982; 54: 2618–20.
22. Hafez I, Kisler K, Berberian K, Dernick G, Valero V, Yong MG, Craighead HG, Lindau M. Electrochemical imaging of fusion pore openings by electrochemical detector arrays. Proc Natl Acad Sci USA 2005; 102: 13879–84.
23. Schroeder TJ, Borges R, Finnegan JM, Pihel K, Amatore C, Wightman RM. Temporally resolved, independent stages of individual exocytotic secretion events. Biophys J 1996; 70: 1061–8.
24. 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. The crucial role of chromogranins in storage and exocytosis revealed using chromaffin cells from chromogranin A null mouse. J Neurosci 2008; 28: 3350–8.
25. Pihel K, Travis ER, Borges R, Wightman RM. Exocytotic release from individual granules exhibits similar properties at mast and chromaffin cells. Biophys J 1996; 71: 1633–40.
26. Marszalek PE, Markin VS, Tanaka T, Kawaguchi H, Fernandez JM. The secretory granule matrix-electrolyte interface: a homologue of the p-n rectifying junction. Biophys J 1995; 69: 1218–29.
27. Borges R, Machado JD, Alonso C, Brioso MA, Gomez JF. Functional role of chromogranins. The intragranular matrix in the last phase of exocytosis. Adv Exp Med Biol 2000; 482: 69–81.
28. Ardiles AO, Maripillan J, Lagos VL, Toro R, Mora IG, Villarroel L, Ales E, Borges R, Cardenas AM. A rapid exocytosis mode in chromaffin cells with a neuronal phenotype. J Neurochem 2006; 99: 29–41.
29. 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. Hypertension from targeted ablation of chromogranin A can be rescued by the human ortholog. J Clin Invest 2005; 115: 1942–52.
30. Colliver TL, Pyott SJ, Achalabun M, Ewing AG. VMAT-mediated changes in quantal size and vesicular volume. J Neurosci 2000; 20: 5276–82.
31. Obermuller S, Calegari F, King A, Lindqvist A, Lundquist I, Salehi A, Francolini M, Rosa P, Rorsman P, Huttner WB, Barg S. Defective secretion of islet hormones in chromogranin-B deficient mice. PLoS One 2010; 5: e8936.
32. Diaz-Vera J, Morales YG, Hernandez-Fernaud JR, Camacho M, Montesinos MS, Calegari F, Huttner WB, Borges R, Machado JD. Chromogranin B gene ablation reduces the catecholamine cargo and decelerates exocytosis in chromaffin secretory vesicles. J Neurosci 2010; 30: 950–7.
33. Diaz-Vera J, Camacho M, Machado J, Dominguez N, Montesinos M, Hernandez-Fernaud J, Lujan R, Borges R. Chromogranins A and B are key proteins in amine accumulation but the catecholamine secretory pathway is conserved without them. FASEB J 2012; 26: 430–8.
34. Dominguez N, Estevez-Herrera J, Pardo MR, Pereda D, Machado JD, Borges R. The functional role of chromogranins in exocytosis. J Mol Neurosci 2012; 48: 317–22.
35. Kopell WN, Westhead EW. Osmotic pressures of solutions of ATP and catecholamines relating to storage in chromaffin granules. J Biol Chem 1982; 257: 5707–10.