Subcellular distribution of chromogranins A and B in bovine adrenal chromaffin cells

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Abstract The major secretory granule proteins chromogranins A (CGA) and B (CGB) have recently been shown to play critical roles in inositol 1,4,5-trisphosphate-dependent intracellular Ca²⁺ mobilizations. We determined here the subcellular distribution of CGA and CGB based on 3D-images of chromaffin cells, and found that ~95% of cellular CGA was present in secretory granules while ~5% was in the endoplasmic reticulum (ER), whereas ~57% of cellular CGB was in secretory granules while ~24% and ~19% were in the ER and nucleus, respectively. These results suggest that chromogranins are at the center of intracellular Ca²⁺ homeostasis in secretory cells.

Keywords: Chromogranin A; Chromogranin B; Chromaffin cell; 3D view; Secretory granule

1. Introduction

Chromogranins A and B (CGA and CGB) are major proteins of secretory granules that contain 40 mM calcium [1–3], and have recently been shown to induce secretory granule formation in both neuroendocrine and non-neuroendocrine cells in which they are expressed [4–6]. Most (>99.9%) of the intragranular calcium is bound to chromogranins in secretory granules [7], and only a very small portion (<0.1%) exists as free Ca²⁺ [7–10]. In accordance with the Ca²⁺ storage roles, CGA binds 32–55 mol of Ca²⁺/mol with a dissociation constant (K_d) of 2.7–4 mM [11], while CGB binds ~90 mol of Ca²⁺/mol with a K_d of 1.5 mM [12].

Further, CGA and CGB interact with the inositol 1,4,5-trisphosphate receptor (IP₃Rs) directly at the intragranular pH 5.5, but at pH 7.5, CGA dissociates from the IP₃R, whereas CGB remains bound to it albeit at a reduced level [13]. This interaction was shown to change the properties of the IP₃R/Ca²⁺ channel significantly; CGA increased the mean open time and the open probability of the IP₃R/Ca²⁺ channel 12- and 9-fold, respectively, in the coupled state [14]. This channel-activating property of CGA persisted as long as CGA remained bound to the IP₃R/Ca²⁺ channel, but disappeared completely when CGA dissociated from the IP₃R/Ca²⁺ channel [14,15]. Similarly, CGB also increased the mean open time and the open probability of the IP₃R/Ca²⁺ channel 42- and 16-fold, respectively [16]. But unlike CGA, which dissociated from the IP₃R/Ca²⁺ channel at pH 7.5 and thus failed to activate the channel, the channel-activating effect of CGB at pH 7.5 was as strong as that at pH 5.5 [16].

The critical contribution of chromogranins in IP₃-dependent intracellular Ca²⁺ mobilization has been shown with both neuroendocrine PC12 and non-neuroendocrine NIH3T3 cells [17]. Expression of chromogranins in NIH3T3 cells increased the IP₃-mediated Ca²⁺ release by 40–134%, whereas suppression of chromogranin expression in PC12 cells decreased it by 40–69% [17]. The chromogranin expression in NIH3T3 cells induced not only secretory granule formation but also expression of the IP₃Rs in the newly formed secretory granules, leading to the increase in the IP₃-mediated Ca²⁺ mobilization in these cells [17].

Despite the proven crucial importance of chromogranins in controlling IP₃-dependent intracellular Ca²⁺ release [18], accurate information about the overall distribution and the concentration of chromogranins in subcellular organelles is lacking. Moreover, it was recently shown that a putative vesicular IP₃-sensitive nucleoplasmic Ca²⁺ store contains both the IP₃Rs and CGB [19], thus further pointing out the potential importance of CGB-IP₃ coupling in the IP₃-dependent Ca²⁺ control mechanisms in the nucleus. It was therefore of utmost importance to know the concentrations of chromogranins in each organelle and the cytoplasmic volumes occupied by these organelles in order to accurately estimate the relative contribution of each organelle in the overall Ca²⁺ homeostasis of the cell. We have analyzed the serial sections of 23 representative bovine chromaffin cells, 27–30 serial sections per cell, using 3D-image reconstruction method, and determined the concentrations of CGA and CGB in each organelle.

2. Materials and methods

2.1. Antibodies

Polyclonal anti-rabbit CGA and CGB antibodies were raised against intact bovine CGA and CGB, and affinity purified on each immobilized purified chromogranin [20].

Abbreviations: CGA, chromogranin A; CGB, chromogranin B; IP₃R, inositol 1,4,5-trisphosphate receptor

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2.2. Immunocytochemical localization of CGA and CGB in serially sectioned bovine chromaffin cells

To obtain 3D images, and for immunogold electron microscopic study of chromaffin cells, tissue samples from bovine adrenal medulla were fixed for 2 h at 4°C in PBS containing 0.1% glutaraldehyde, 4% paraformaldehyde, and 3.5% sucrose. After three washes in PBS, the tissues were postfixed with 1% osmium tetroxide on ice for 2 h, washed three times, and stained en bloc with 0.5% uranyl acetate, all in PBS. The tissues were then embedded in Epon 812 after dehydration in an ethanol series. A ribbon of 120–140 consecutive serial sections in 70-nm thickness was made from the embedded tissue and placed on the surface of water. The ribbon was then manipulated so that 6–7 sections/grid were successively picked up on Formvar/carbon-coated nickel grids. As the ribbon was picked up, the region of interest was carefully positioned so that it was located over the grid openings and not on a grid bar. For immunogold labeling, the serial sections that had been transferred to grids were floated on drops of freshly prepared 3% sodium metaperiodate for 30 min, and followed the procedure described before [20].

2.3. 3D-image construction of chromaffin cells and subcellular organelles

For 3D-image construction, every 4–5 section, representing one section per every 400–500 nm of cytoplasm, was chosen from 120 to 140 consecutive serial sections per cell, and the resulting ~27–30 serial TEM images were used in reconstructing the 3D image of each cell. The outlines of the structures of interest were manually traced and transformed into digitized form. After z-axis alignment of the digitized 27–30 image stacks, 3D reconstruction of digitized contours was obtained and rendered by using 3Dmax 6.0 software (Discreet Logic Inc., Canada). A representative 2D image of a chromaffin cell from which the 3D images are generated was obtained from one of the middle sections of the cell, and the location of the CGA- or CGB-labeling immunogold particles was marked with yellow dots. Other details are as described in [21].

3. Results

3.1. 3D-image of the cell

To obtain 3D-images of chromaffin cells and to determine the total amount of CGA and CGB present in these cells, 23 whole bovine adrenal medullary chromaffin cells were serially sectioned, 27–30 serial sections per cell, from one end of the cell to the other end (Figs. 1A and 2A), and these serial sections were treated with CGA- or CGB-labeling gold particles for immunogold electron microscopy (Fig. 1B and 2B). Figs. 1C and 2C are the line drawings of Figs. 1A and 2A, respectively, which represent the central sections of two separate cells. The rough see-through 3D images of whole cells were obtained from these line images (Fig. 3). Unlike results obtained from a limited number of random sections of the same cell, present results obtained from 23 cells with ~27–30 serial sections per cell allowed us to obtain not only the 3D-reconstruction of chromaffin cells showing secretory granules, the ER, mitochondria, and the nucleus (Fig. 3), but also the relative concentrations of CGA or CGB in each organelle.

3.2. Nucleus, endoplasmic reticulum and mitochondria

The shape and size of the chromaffin cells were not uniform and differed from cell to cell. Cells that are located near the blood vessel (Fig. 4A, “a” type cells) were larger and more elongated compared to those that are more distant from the duct (Fig. 4A, “b” type cells). Reflecting different shapes and sizes of the cells, the cell volumes as well as the volumes occupied by the subcellular organelles varied greatly (Fig. 4B). The ER was found to localize throughout the cytoplasm, not showing any tendency to cluster (Fig. 3A). The cell volume occupied by the nucleus and ER varied from one cell to another.

3.3. Secretory granules

The number and subcellular location of secretory granules differed significantly depending on the location of the cell within the adrenal gland (Fig. 4). The number of secretory granules was generally higher in cells that are located near the adrenal gland duct (Fig. 4A, “a” type cells) than in cells that are more distant from the duct (Fig. 4A, “b” type). Moreover, in cells that are located near the duct there was a clear tendency for secretory granules to localize preferentially near the plasma membrane, heavily clustering in the side of the cytoplasm that faces the duct. As evident in Fig. 4A and in the 3D image of an “a” type cell shown in Fig. 3, in cells of this type the nucleus appears to be displaced toward the opposite side of the granules in the cytoplasm. However, in cells that are distant from the duct (Fig. 4A, “b” type cells) the nucleus is more or less in the center of the cytoplasm, while secretory granules appeared to be scattered randomly around the nucleus. To determine the number of secretory granules in chromaffin cells, we counted all secretory granules present in each electron micrograph and using a conversion factor of 2.27 (see below) extrapolated the numbers to the whole cell. Since each electron micrograph that had been used to count secretory granules represents one section per every ~500 nm of cytoplasm and the average diameter of each granule is ~300 nm, it was estimated that a half of secretory granules that are present in a chromaffin cell would be absent in the electron micrographs used in the counting, thereby resulting in the presence of only ~50% of the total granules in the electron micrographs. Moreover, even in the electron micrographs where the sectioned secretory granules are present, the image of secretory granule would not be clear enough to be counted as secretory granules unless the granules are sectioned sufficiently inside the granule boundaries. We estimated that the image of secretory granules would not be clearly discernible unless the section is at least 30 nm inside the boundary of granules, leading to detection of secretory granules in ~88% of the case, i.e. detection of granules in the 440 nm span of 500 nm cytoplasm. Combining this number and the above detection limit (50%), we estimate that the number of secretory granules counted in the electron micrographs represents only 1 out of 2.27 granules present in a cell, thus giving a conversion factor of 2.27. Therefore, we multiplied the number of secretory granules counted in 27–30 electron micrographs/cell with 2.27 to come up with the total number of secretory granules in a chromaffin cell. The number of secretory granules present in each cell varied widely from one cell to another, ranging from 7300 to 41 400 per cell, with an average of 23 500 ± 10 100 (mean ± S.D., n = 23). In cells that are near the blood vessel in the adrenal gland (Fig. 4A, “a” type cells), there were >30 000 secretory granules per cell, compared to significantly less granules per cell in those that are located some distance away from the blood vessel (Fig. 4A, “b” type cells). The larger number of secretory granules in cells that line the duct is in line with the physiological function of those cells to secrete the granule contents.

3.4. Volume of the cell

From the 3D images, the total volume of chromaffin cells was calculated to be 1998 ± 496 μm³ (mean ± S.D., n = 23).
Since the size of each chromaffin cell varied greatly, the cell volume also varied, ranging from 1100 to 3180 \( \mu m^3 \) (Fig. 4B). To determine the volumes occupied by each organelle in the cell, the areas occupied by each organelle were measured in relation to the areas occupied by the whole cell from all 27–30 serial EM images, and this was repeated for all of the 23 cells. The sum of the areas of each organelle was then divided by that of total cell to estimate the percentage of volume occupied by each organelle. The nucleus was estimated to occupy 16.7 ± 3.8% (mean ± S.D., \( n = 23 \)) of the cell volume while secretory granules 20.5 ± 5.2% (mean ± S.D., \( n = 23 \)). The ER occupied 14.2 ± 3.5% (mean ± S.D., \( n = 23 \)) of the cell volume, whereas the mitochondria 2.9 ± 1.2% (mean ± S.D., \( n = 23 \)). Since the areas of each organelle were measured from all 23 cells, with 27–30 serial EM images per cell, the percentages obtained for each organelle will accurately reflect the actual volume occupied by these organelles in the cell.

3.5. Distribution and concentration of CGA

To determine the relative concentrations of CGA in each organelle, the CGA-labeling immunogold EM study was carried out using all the serial sections of 5 chromaffin cells, a total of 140 electron micrographs (Fig. 1B and Table 1). The presence of CGA in secretory granules and endoplasmic reticulum is evident (Fig. 1B). However, as shown previously, CGA is absent from the mitochondria [20]. The gold particles found in each organelle of 5 chromaffin cells (140 electron micrographs) were counted and the area of each organelle from all
these sections was summed to obtain the total number of CGA-labeling gold particles and the total area of each organelle (Table 1).

As shown in Table 1, the number of CGA-labeling gold particles found per \( \mu m^2 \) of the ER was 1.8, while that of secretory granules was 18.5. The number of gold particles found per \( \mu m^2 \)
of the nucleus was 0.5, which was similar to 0.4 of mitochondria (non-specific background), indicating the absence of CGA in the nucleus. This result demonstrates that CGA localizes exclusively to secretory granules and the endoplasmic reticulum, but not in the nucleus and mitochondria, consistent with our previous results [20]. In view of the fact that the bovine chromaffin granules contain ~2 mM chromogranins and ~90% of this is CGA [22,23], the CGA concentration in secretory granules is estimated to be ~1.8 mM and that of the ER ~0.14 mM. Hence, considering the cell volumes occupied by secretory granules and the ER, and the relative CGA concentrations in these organelles, it is estimated that ~95% of cytoplasmic CGA exists in secretory granules and ~5% in the ER (Table 1).

Table 1
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Five cells, 140 electron micrographs, from three different tissue preparations were used.

Likewise, the CGB-labeling immunogold EM study was also carried out using all the serial sections of 5 chromaffin cells, a total of 150 electron micrographs (Fig. 2B and Table 2). Similar to CGA, CGB was present in secretory granules and the ER, but not in mitochondria. In addition, CGB also localized in the nucleus (Fig. 2B), as has been reported before [20]. As shown in Table 2, the number of CGB-labeling gold particles found per μm² of the ER was 5.5, while those of secretory granules and the nucleus were 8.4 and 4.0, respectively. The number of gold particles found per μm² of mitochondria was 0.8, which indicates the level of non-specific background. Hence, from the fact that the number of gold particles found per μm² of mitochondria was 0.8, which indicates the level of non-specific background. These results indicate that the CGB concentration in the ER is ~60% that of secretory granules. Hence, from the fact that

Table 2
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Five cells, 150 electron micrographs, from three different tissue preparations were used.

3.6. Distribution and concentration of CGB

Likewise, the CGB-labeling immunogold EM study was also carried out using all the serial sections of 5 chromaffin cells, a total of 150 electron micrographs (Fig. 2B and Table 2). Similar to CGA, CGB was present in secretory granules and the ER, but not in mitochondria. In addition, CGB also localized in the nucleus (Fig. 2B), as has been reported before [20]. As shown in Table 2, the number of CGB-labeling gold particles found per μm² of the ER was 5.5, while those of secretory granules and the nucleus were 8.4 and 4.0, respectively. The number of gold particles found per μm² of mitochondria was 0.8, which indicates the level of non-specific background. These results indicate that the CGB concentration in the ER is ~60% that of secretory granules. Hence, from the fact that

Fig. 4. Electron micrograph of bovine adrenal medulla and distribution profile of the total cell volumes of chromaffin cells. (A) An electron micrograph of bovine adrenal medulla, showing different types of chromaffin cells, is shown. Cells that line the blood vessel (BV) are large and elongated with many secretory granules (“a” type cells) while cells that are distant from the blood vessel are small and contain relatively smaller number of secretory granules (“b” type cells). Another “b” type cell is shown in the inset. Bar = 2 μm. (B) Distribution profile of the total cell volumes of 23 bovine adrenal medullary chromaffin cells is shown. The average volume of 1998 ±496 (mean ± S.D., n = 23) μm³ is written in the top right corner.
the bovine chromaffin granules contain ~2 mM chromogranins and ~10% of this is CGB [22,23], the CGB concentration in secretory granules is estimated to be ~200 μM. Considering that the CGB concentration in the ER is ~60% that of secretory granules, the CGB concentration in the ER is estimated to be ~120 μM. Likewise, the CGB concentration in the nucleus is estimated to be ~80 μM.

Taking the cell volumes occupied by each organelle, it is estimated that secretory granules contain ~57% of total cellular CGB, while the ER and nucleus contain ~24% and ~19%, respectively (Table 2). In light of the fact that CGB is one of the major intragranular matrix proteins, traditionally referred to as a marker protein of secretory granules [24], it is remarkable that the nucleus contains a substantial amount of cellular CGB. In stark contrast to CGA, which does not exist in the nucleus, the presence of a substantial amount of CGB in the nucleus implies important roles of nuclear CGB in addition to its reported role in transcriptional regulation [20]. Combined with the high capacity Ca2+-binding property of CGB and the presence of all three isoforms of IP3R in the nucleoplasm [25], the presence of large amounts of CGB in the nucleus suggests key roles for nuclear CGB in controlling the intranuclear Ca2+ concentration.

4. Discussion

Present results show that CGA concentrations in secretory granules and the ER are ~1.8 mM and 0.14 mM, respectively (Table 1), whereas CGB concentrations in secretory granules and the ER are ~200 μM and ~120 μM, respectively (Table 2). In addition, CGB was estimated to be present in the nucleus at ~80 μM CGB (Table 2). This means that ~95% cellular CGA exists in secretory granules and ~5% exists in the ER, whereas ~57% cellular CGB exists in secretory granules while ~24% and ~19% are present in the ER and nucleus, respectively. In view of the high capacity Ca2+-storage property and the IP3R/Ca2+ channel activating roles of chromogranins A and B [14,16], selective localization of chromogranins in subcellular organelles suggests the importance of these organelles in the control of cytoplasmic Ca2+ concentration. In this regard, the amount and concentration of chromogranins in these organelles are likely to indicate the relative contribution of each organelle in the control of cytoplasmic Ca2+ concentration.

Moreover, presence of CGB in the nucleus and ER points to the potential importance of CGB in controlling IP3-dependent intracellular Ca2+ release in these organelles. Using neuronally differentiated PC12 cells it has recently been shown that coupling of CGB or CGA/CGB mixture to the IP3R/Ca2+ channel in the ER is a necessary component for IP3-dependent Ca2+ release mechanism in the ER [18]. This result suggested that the IP3R-chromogranin complex in the ER functions as major components of a basic functional IP3R/Ca2+ channel unit. Even in the case of the nucleus, it appears highly likely that the IP3R-CGB complex also functions as a basic functional Ca2+ channel unit, given the presence of the IP3Rs in the nucleus [25] and the near physiological pH of the nucleus.

Recently, we have determined the distribution and relative concentrations of the IP3Rs in subcellular organelles in chromaffin cells [21]. The ER of bovine chromaffin cells was shown to contain 15–20% of each of the three IP3R isoforms while secretory granules contained 58–69%. Reflecting the large amounts of IP3Rs in secretory granules, the relative concentrations of the IP3Rs in secretory granules were at least 2-3-fold higher than those of the ER and nucleus [21]. Moreover, the nucleus was estimated to contain ~15% each of IP3R-1 and -2, but 25% of IP3R-3. The IP3R concentrations of nucleus were not much different from those of the ER; those of IP3R-1 and -2 were slightly lower than those of the ER but that of IP3R-3 was slightly higher [21]. In view of the dearth of knowledge regarding the IP3-dependent Ca2+ control mechanisms in the nucleus, the abundance of the IP3Rs in the nucleus came as a surprise and suggested the importance of the IP3-dependent Ca2+ control mechanisms in the nucleus.

Furthermore, we found in a recent study the presence of a vesicular nucleoplasmic structure that is composed of the IP3Rs, chromogranin B, and phospholipids [19]. This vesicular complex contained all three isoforms of the IP3R, in addition to chromogranin B and phosphatidylinositol 4,5-bisphosphate, in a structure with an estimated mass of ~2–3 x 10⁵ daltons [19]. In our preliminary studies, these structures appeared to release Ca2+ in response specifically to IP3, but not to inositol 1,4-bisphosphate and inositol 1,3,4,5-tetakisphosphate, suggesting that these structures are IP3-sensitive vesicular nucleoplasmic Ca2+ stores. Given the presence of large amounts of both IP3Rs and CGB in the nucleus, this putative IP3-sensitive vesicular nucleoplasmic Ca2+ store might be able to play a major role in controlling the IP3-dependent intranuclear Ca2+ concentration.

The predominant presence of both the IP3R/Ca2+ channels and the Ca2+ storage proteins chromogranins A and B in secretory granules is thought to underscore the importance of secretory granules in the IP3-dependent Ca2+ control mechanisms in the cell. In this regard, the function of secretory granules in secretory cells (neurons, exo/endocrine, and neuroendocrine cells) appears to be far beyond a mere storage of the secretory contents. Secretory granules contain not only the secretory contents, such as hormones and neurotransmitters, but also a majority of the cellular Ca2+, along with the IP3R/Ca2+ channels and chromogranins. This means that secretory granules contain basically all the essential components that are necessary in initiating and executing the exocytotic processes. Thus, combined with the presence of 40 mM Ca2+ and the highest chromogranin concentrations in secretory granules (Tables 1 and 2), the present results highlight the prominence of secretory granules and chromogranins in the calcium world of secretory cells through the participation of the IP3R/Ca2+ channels.

Since the secretory process is initiated by a sudden increase in intracellular Ca2+ concentrations in response to IP3-triggered Ca2+ release [26,27], and secretory granules serve as a major IP3-sensitive intracellular Ca2+ store [28], the large number of secretory granules in the release-ready cells will serve perfectly well the dual needs of the cells both to experience a sudden increase of intracellular Ca2+ concentrations and to secrete the granule contents.

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References

[8] Gerasimenko, O.V., Gerasimenko, J.V., Belan, P.V. and Petersen,
[19] Yoo, S.H., Nam, S.W., Huh, S.K., Park, S.Y. and Huh, Y.H.
receptor/Ca2+ channel modulatory role of chromogranin a, a
Ca2+ storage protein of secretory granules. J. Biol. Chem. 275,
15067–15073.


(2005) Effects of chromogranin expression on inositol 1,4,5-
trisphosphate-induced intracellular Ca2+ mobilization. Biochem-
istry 44, 6122–6132.

Functional coupling of chromogranin with the inositol 1,4,5-
trisphosphate receptor shapes calcium signaling. J. Biol. Chem. 279, 35551–35556.

[19] Yoo, S.H., Nam, S.W., Huh, S.K., Park, S.Y. and Huh, Y.H.
(2005) Presence of a nucleoplasmic complex composed of the
inositol 1,4,5-trisphosphate receptor/Ca2+ channel, chromogranin B, and phospholipids. Biochemistry 44, 9246–9254.

protein chromogranin B in the nucleus: potential role in

Distribution profile of inositol 1,4,5-trisphosphate receptor iso-


and B: the first 25 years and future perspectives. Neuroscience 49,
497–528.

(chromogranin/secretogranin) family. Trends Biochem. Sci. 16,
27–30.

trisphosphate receptor isoforms in the nucleoplasm. FEBS Lett.
555, 411–418.

high calcium concentration in a presynaptic terminal. Science 256,
677–679.

[27] Heidelberger, R., Heinemann, C., Neher, E. and Matthew, G.

channel. J. Biol. Chem. 275, 35551–35556.


[30] Gerasimenko, O.V., Gerasimenko, J.V., Belan, P.V. and Petersen,
O.H. (1996) Inositol trisphosphate and cyclic ADP-ribose-med-
iated release of Ca2+ from single isolated pancreatic zymogen


dent luminal oscillations and release of Ca2+ and H+ from mast
cell secretory granules: Implications for signal transduction.
Biophys. J. 85, 963–970.

Ca2+-binding of Chromogranin A: Relationship between the pH-
induced conformational change and Ca2+ binding property. J.
Biol. Chem. 266, 7740–7745.

[34] Yoo, S.H., Oh, Y.S., Kang, M.K., Huh, Y.H., So, S.H.,
subtypes of the inositol 1,4,5-trisphosphate receptor/Ca2+
channel in the secretory granules and coupling with the
Ca2+ storage proteins chromogranins A and B. J. Biol.
Chem. 276, 45806–45812.

Jeon, C.J. (2000) Coupling of the inositol 1,4,5-trisphosphate
receptor and chromogranins A and B in secretory granules. J.
Biol. Chem. 275, 12553–12559.