Brief Communication

Chromogranin A Deficiency in Transgenic Mice Leads to Aberrant Chromaffin Granule Biogenesis

Taeyoon Kim,[1] Chun-fa Zhang,[1] Ziqing Sun,[2] Heling Wu,[2] and Y. Peng Loh[1]

[1]Section on Cellular Neurobiology, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892, and [2]College of Biology, Peking University, 100871 Beijing, China

The biogenesis of dense-core secretory granules (DCGs), organelles responsible for the storage and secretion of neurotransmitters and neuropeptides in chromaffin cells, is poorly understood. Chromogranin A (CgA), which binds catecholamines for storage in the lumen of chromaffin granules, has been shown to be involved in DCG biogenesis in neuroendocrine PC12 cells. Here, we report that downregulation of CgA expression *in vivo* by expressing antisense RNA against CgA in transgenic mice led to a significant reduction in DCG formation in adrenal chromaffin cells. The number of DCGs formed in CgA antisense transgenic mice was directly correlated with the amount of CgA present in adrenal medulla. In addition, DCGs showed an increase in size, with enlargement in the volume around the dense core, a phenomenon that occurs to maintain constant "free" catecholamine concentration in the lumen of these granules. The extent of DCG swelling was inversely correlated with the number of DCGs formed, as well as the amount of CgA present in the adrenal glands of CgA antisense transgenic mice. These data indicate an essential role of CgA in regulating chromaffin DCG biogenesis and catecholamine storage *in vivo*.

Key words: **chromogranin A; dense-core; secretory granule; biogenesis; transgenic; chromaffin; catecholamine; adrenal medulla**

Introduction

Catecholamines (CAs), the major neurotransmitter in adrenal chromaffin cells, are involved in the regulation of physiological processes and implicated in neurologic, endocrine, and cardiovascular diseases. Newly synthesized CAs are stored and secreted from dense-core secretory granules (DCGs) in adrenal chromaffin cells (Albillos et al., 1997). Active sequestration of newly synthesized CAs into DCGs is mediated by the vesicular monoamine transporter (Erickson et al., 1992), which also keeps the concentration of CAs in DCGs constant by balancing out the passive leakage of CAs from these organelles (Eisenhofer et al., 2004). In the lumen of DCGs, CAs bind saturably to chromogranin A (CgA), an acidic glycoprotein abundant in adrenal chromaffin granules (Westermann et al., 1988; Videen et al., 1992). Therefore, effective storage of CAs in adrenal medullary cells is expected to depend on the quantity of CgA available for CA binding, as well as the number of DCGs formed in the cells.

The understanding of the physical and regulatory mechanisms governing DCG biogenesis is only at its infancy. It has been proposed that aggregation of granule cargo proteins provides a mechanical force to drive the formation of DCGs. Granule cargo proteins such as granins (e.g., CgA, secretogranin I, and secretogranin II) and hormones have been shown to aggregate under low pH and high calcium concentration *in vitro*, conditions present in the lumen of DCGs (Yoo and Albanesi, 1990; Chanat and Huttner, 1991; Colomer et al., 1996; Yoo, 1996). Indeed, granins and other granule proteins transfected into fibroblasts have been shown to induce formation of DCGs (Kim et al., 2001; Huh et al., 2003; Beuret et al., 2004). Recently, regulatory proteins involved in controlling the quantity of DCGs formed have been identified. Polypyrimidine tract-binding protein was shown to stabilize mRNAs encoding granule proteins after glucose stimulation, thereby increasing the amount of these proteins, which then induced the formation of more insulin-containing DCGs in pancreatic β cells (Knoch et al., 2004). In another study, CgA was shown to regulate the formation of DCGs in catecholamine-containing PC12 cells (Kim et al., 2001), possibly by protecting granule proteins from degradation, and therefore increase their availability for DCG formation (Kim et al., 2003). CgA may play a more important role in regulating DCG biogenesis than being just a granulogenic aggregative protein.

Thus far, DCG biogenesis in neuroendocrine cells has only been studied in cell lines. To determine whether CgA plays a role in DCG biogenesis *in vivo*, we generated transgenic (TG) mice that were downregulated in CgA biosynthesis by expressing the antisense sequence against CgA. In this study, we demonstrate, by electron microscopy (EM) using these partially CgA-deficient mice, that CgA plays a pivotal role in determining the number of dense-core chromaffin granules formed in a dose-dependent manner.

Materials and Methods

Construction of CgA gene antisense vector. A plasmid (pCAS2C) containing CgA antisense sequence (−83 to 219 of mouse CgA cDNA sequence)

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Correspondence should be addressed to Y. Peng Loh, 49 Convent Drive, MSC-4480, Bethesda, MD 20892. E-mail: lohp@mail.nih.gov.

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under the control of the endogenous CgA promoter for the tissue-specific expression of mouse CgA antisense fragments was previously constructed and validated (Liu et al., 2001).

Generation of transgenic mice. Fertilized one-cell embryos were obtained by mating between superovulated female mice (a hybrid of C57BL/6N and C3H) and male mice. The 2.9 kb fragment containing the CgA promoter, a CgA antisense (CgA–AS), chloroamphenicol acetyltransferase and phosphoglycerate kinase 1 poly(A) sequences (see Fig. 1*A*) was separated from pCAS2C by *Eco*RI digestion and isolated by gel purification. The resulting fragment was microinjected into pronuclei. Of 82 injections, 18 positive founder mice were obtained. PCR amplification was used to identify the CgA–AS transgene. To obtain a higher dosage of CgA–AS transgene, a pair of CgA–AS transgene positive founders was mated, and five pups; three males and two females) were born. All were positive for the CgA–AS transgene. One male (TG-1) and two female (TG-4 and TG-5) animals along with two wild-type (WT) control mice [male (WT-1) and female (WT-2)] were analyzed in this study.

Identification of CgA–AS transgene. Tail genomic DNA was isolated using NucleoSpin Tissue kit (Qiagen, Valencia, CA). Two primers, C1 (5′-ATGGAGAAAAAAATCACTGGATATACC-3′) and P1 (5′-GACTCGAGCTTTGGTAGCCAGACTACAG-3′), were used for PCR screening (~520 bp PCR product was amplified from 35 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 30 s).

Western blotting analysis. Each adrenal gland from CgA–AS transgenic or wild-type mice was lysed in the lysis buffer containing protease inhibitor mixture (Roche Diagnostics, Indianapolis, IN). The other gland was used for EM morphometry (see below). Lysates were mixed with an equal volume of 2× SDS sample buffer. An equal amount of each sample was loaded onto 12% Tris-glycine precast gels (Invitrogen, Carlsbad, CA), and proteins were transferred onto nitrocellulose membranes. The membranes were blocked in 5% nonfat milk in PBS at room temperature for 1 h and then incubated overnight with primary antibodies at 4°C. After washing, the membrane was incubated with secondary antibody conjugated with HRP at room temperature for 1.5 h. Subsequently, chemiluminescent substrate (SuperSignal West Pico; Pierce, Rockford, IL) was used for the detection of signal. Anti-CgA rabbit polyclonal antibody (TK-1) (Arnaoutova et al., 2003), anti-chromogranin B (CgB) mouse monoclonal antibody (Chemicon, Temecula, CA), and anti-carboxypeptidase E (CPE) rabbit polyclonal antibody (Cool et al., 1997) were used at 1:5000 (CgA and CgB) and 1:3000 (CPE). For control, a Golgi marker, Vti1a, was analyzed using an anti-Vti1a monoclonal antibody (BD Biosciences, San Jose, CA) at 1:2500.

EM morphometry and statistics. Adrenal glands were dissected and fixed in 2.5% glutaraldehyde. The fixed glands were processed for EM at JFE Enterprises (Brookeville, MD). For quantitative measurement of DCGs, each EM micrograph was printed at the final magnification of 30,000, scanned, and converted into digital images. The number of DCGs and the cytoplasmic area less nucleus were measured from each EM digital image, and the average number of DCGs per square micrometer of cytoplasm, their diameters, and the diameters of dense cores were analyzed with MetaMorph 6.0 (Universal Imaging, Downingtown, PA) and Excel (Microsoft, Redmond, WA). The total cytoplasmic area of each micrograph was obtained by tracing the area using Intuos2 Graphics Tablet (Wacom, Vancouver, WA). Student's *t* test was used for all statistical calculations (two-tailed analysis). Average cytoplasmic areas obtained from EM micrographs were 52.23 ± 0.49 (WT-1; $n = 11$), 45.34 ± 1.88 (WT-2; $n = 5$), 49.77 ± 1.28 (TG-1; $n = 12$), 49.63 ± 1.50 (TG-4; $n = 15$), and 48.71 ± 3.14 μm^2 (TG-5; $n = 9$) per image.

Results

Reduction of CgA levels in CgA–AS transgenic mice

A plasmid, pCAS2C, containing a mouse CgA sequence in the antisense direction (−83 to 219 of mouse CgA mRNA) under the mouse CgA promoter, was constructed previously, and its expression was validated in transgenic animals (Fig. 1*A*) (Liu et al., 2001). Founders expressing CgA–AS transgene were obtained. PCR amplification using a unique primer set (C1 and P1) amplifying a ~520 bp product was detected only in CgA–AS transgenic mice and plasmid control but not in wild-type animals (Fig. 1*A*). Western blotting analysis for CgA using adrenal glands from three CgA–AS transgenic mice (TG-1, TG-4, and TG-5) showed significant reduction of CgA levels to the range of 30–70% of the wild-type control animals (WT-1 and WT-2). Compared with WT-1 (100%), the level of CgA in TG-1 was reduced to 51% (Fig. 1*C*). Compared with WT-2 (100%), the levels of CgA were reduced to 68% (TG-4) and 31% (TG-5). TG-5 showed the most significant reduction in CgA protein level (Fig. 1*C*). We also examined the levels of other granule proteins such as CgB and CPE. As shown in Figure 1*B*, these granule proteins were also decreased in their levels in the CgA–AS transgenic mice. However, the level of a nongranule protein, Vti1a, a Golgi marker protein, was not decreased in the transgenic mice relative to the control (data not shown).

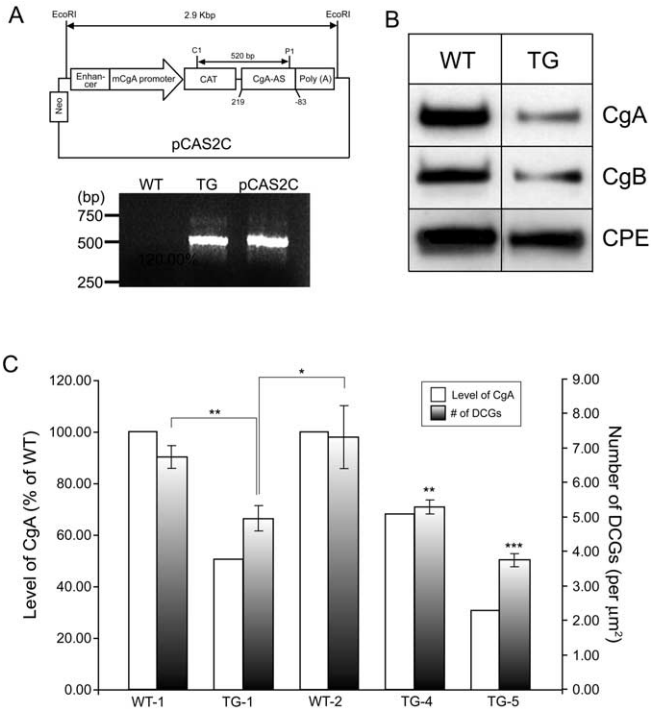

Figure 1. Reduction of DCG formation in CgA antisense transgenic mice. ***A***, Diagram of CgA antisense transgene plasmid. A transgenic vector (pCAS2C) containing mouse CgA antisense sequence under the control of the endogenous mouse CgA promoter for the tissue-specific expression was generated previously (Liu et al., 2001). PCR amplification using a specific primer set (C1 and P1) with genomic DNAs from WT, transgenic animals, and a plasmid (pCAS3C) is shown. Only TG and a plasmid amplification showed PCR product at ~520 bp. ***B***, Western blotting analysis representing the level of CgA, CgB, and CPE in WT and transgenic animals. ***C***, Bar graph represents the level of CgA in adrenal glands (open bars) and the number of DCGs (filled bars). The level of CgA was reduced to 68% (TG-4) and 31% (TG-5) compared with WT-2 (100%). ***C***, The level of CgA in TG-1 was reduced to 51% compared with WT-1. The number of DCGs are 7.3 ± 0.9 (WT-1; $n = 11$ EM images), 6.7 ± 0.3 (WT-2; $n = 5$), 4.6 ± 0.5 (TG-1; $n = 12$; $^{**}p < 0.01$ vs WT-1; $^{*}p < 0.05$ vs WT-2), 5.3 ± 0.2 (TG-4; $n = 15$; $^{**}p < 0.01$ vs WT-2), and 3.8 ± 0.2 (TG-5; $n = 9$; $^{***}p < 0.001$ vs WT-2) granules per square micrometer of cytoplasm (±SEM; two-tailed Student's *t* test).

Decreased number of DCGs in CgA–AS transgenic mice

To quantify the number of granules formed in CgA–AS transgenic mice, we performed morphometric studies using EM. Analysis of the number of DCGs from EM micrographs taken from different areas of adrenal medulla showed significant reduction in CgA–AS transgenic mice compared with wild-type mice.

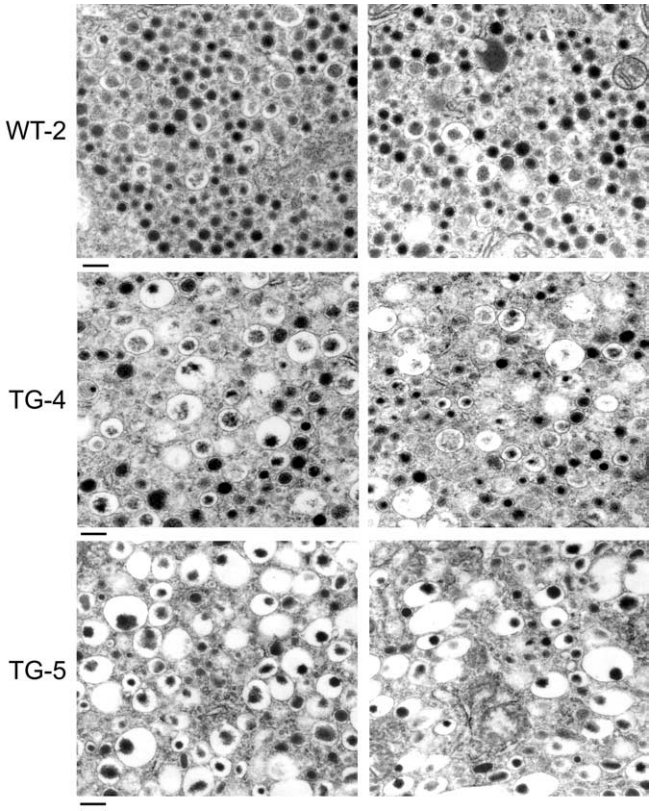

Figure 2. Morphological changes of DCGs in CgA antisense transgenic mice. EM micrographs represent cytoplasmic images from adrenal chromaffin cells in WT-2, TG-4, and TG-5. Two images per animal are shown. Scale bar, 300 nm.

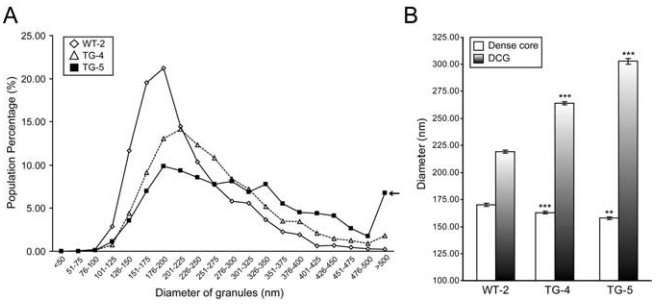

Figure 3. Increased size of DCGs in CgA antisense transgenic mice. ***A***, EM morphometric analysis showing the percentage of DCGs with different diameters. The arrow indicates DCG population with a diameter >500 nm in TG-5. ***B***, Average diameters of DCGs (filled bars) and dense cores (open bars) are shown. Average sizes of DCGs are 219.2 ± 1.4 nm (±SEM; n = 2062), 263.9 ± 1.5 nm (±SEM; n = 3648; ***$p < 0.0001$ vs WT-2), and 302.9 ± 2.6 nm (±SEM; n = 2757; ***$p < 0.0001$ vs WT-2) for WT-2, TG-4, and TG-5, respectively. Average diameters of the dense cores are 170.2 ± 1.4 nm (±SEM; n = 1256), 163.2 ± 1.4 nm (n = 1017; ***$p < 0.0001$ vs WT-2), and 157.9 ± 1.2 nm (n = 1116; **$p < 0.01$ vs WT-2) for WT-2, TG-4, and TG-5, respectively (two-tailed Student's t test).

The number of DCGs from two wild-type control animals were 6.7 ± 0.3 (WT-1; n = 11 EM images) and 7.3 ± 0.9 (WT-2; n = 5 EM images) granules per square micrometer of cytoplasm area of EM micrographs. The number of granules in WT adrenal chromaffin cells was not significantly different between male and female animals. In contrast, three CgA–AS transgenic animals, TG-1, TG-4, and TG-5, showed a significant decrease in numbers of DCGs: 4.6 ± 0.5 (TG-1; n = 12 EM images; $p < 0.01$ vs WT-1; $p < 0.05$ vs WT-2); 5.3 ± 0.2 (TG-4; n = 15 EM images; $p < 0.01$ vs WT-2); and 3.8 ± 0.2 (TG-5; n = 9 EM images; $p < 0.001$ vs WT-2) granules per square micrometer of cytoplasm area of EM micrographs, respectively (Fig. 1*C*). This represents a reduction to 68% (TG-1 vs WT-1), 72, and 51% (TG-4 and TG-5, respectively, vs WT-2). These data show that the amount of CgA present in the adrenal glands of CgA–AS transgenic animals was correlated with the number of DCGs formed, indicating that CgA plays a critical role in regulating quantitative DCG biogenesis in adrenal chromaffin cells *in vivo*.

Enlarged DCGs in CgA–AS transgenic mice

Interestingly, we also observed altered morphology of DCGs in CgA–AS transgenic animals. Different from wild-type adrenal chromaffin cells in which the DCGs showed a membrane tightly surrounding the dense core, CgA–AS transgenic animals showed large numbers of DCGs with increased volume around the core (Fig. 2). When the distribution of DCG populations based on their diameter was analyzed in WT-2 using morphometric analysis, the diameter of most of the WT-2 DCGs (~96%) was in the range of 126–325 nm, with a peak population (~21% of total) at 176–200 nm (Fig. 3*A*). The distribution pattern was significantly different in CgA–AS transgenic mice. TG-4 showed that the diameter of a significant proportion of DCGs was shifted to a peak at 201–225 nm (~14% of total), whereas the majority (~80%) was in the range of 151–350 nm (Fig. 3*A*). Such a diameter shift was most significant in TG-5, the animal with the highest reduction in CgA level and the number of DCGs. In TG-5, no outstanding peak population of DCGs was present, rather the diameter of DCGs was widely distributed in the range of 151–375 nm (~71%) (Fig. 3*A*). Additionally, there was a significant increase in a population of DCGs with a diameter >500 nm (~7% of total) in the TG-5 animal (Fig. 3*A*, arrow). The mean DCG diameter of WT-2, TG-4, and TG-5 was 219.2 ± 1.4, 263.9 ± 1.5, and 302.9 ± 2.6 nm, respectively, showing that the size of DCGs in CgA–AS transgenic mice was inversely correlated to the amount of CgA, as well as the number of DCGs formed in adrenal medulla of CgA–AS transgenic mice (Fig. 3*B*). Therefore, these data indicate that, in CgA deficient mice, not only the number but also the size of DCGs was affected in adrenal chromaffin cells. We also measured the total DCG area and expressed it as a percentage of the total cytoplasmic area for each EM micrograph. The percentage was ~27% for WT-2, ~36% for TG-4, and ~40% for TG-5. This indicates a ~33% (TG-4) and ~48% (TG-5) increase in the area occupied by DCGs in the cells of transgenic animals, relative to the wild-type mouse (WT-2). To determine whether this swelling of DCGs may be contributed by the reduction of core size, we measured the diameter of dense cores formed in the transgenic mice. In a wild-type animal (WT-2), the average size of dense cores was 170.2 ± 1.4 nm (±SEM; n = 1256). In the transgenic mice, TG-4 and TG-5, the average sizes of dense cores were reduced to 163.2 ± 1.4 nm (n = 1017; $p < 0.0001$ vs WT-2) and 157.9 ± 1.2 nm (n = 1116; $p < 0.01$ vs WT-2), respectively (Fig. 3*B*). The reduction in core size was ~4% (TG-4) and ~7% (TG-5) compared with the control animal (WT-2). Such a small reduction in core size would not contribute significantly to the swelling of DCGs in the transgenic mice.

Discussion

CgA controls DCG biogenesis in chromaffin cells *in vivo*

Biogenesis of DCGs in adrenal chromaffin cells is a prerequisite step for the proper storage, processing, and regulated secretion of CAs. Analyses on CgA–AS transgenic animals showed that CgA is a key molecule regulating the number of CA-containing DCGs in

adrenal chromaffin cells *in vivo*. The number of DCGs formed in adrenal chromaffin cells of these animals was significantly decreased in a CgA dose-dependent manner. Thus, these transgenic animal data provide evidence for a role of CgA in DCG formation *in vivo*, which was otherwise suggested previously only from studies in PC12 cells (Kim et al., 2001; Huh et al., 2003). The mechanism for CgA-mediated DCG biogenesis *in vivo* is unknown. Studies using neuroendocrine PC12 and endocrine 6T3 cells suggest that CgA might be involved in the regulation of granule protein stability (Kim et al., 2001, 2003). In CgA-deficient PC12 cells, other granule proteins such as CgB and CPE were degraded (Kim et al., 2001). Consistent with this observation, the levels of CgB and CPE in CgA–AS transgenic mice were decreased (Fig. 1*B*), suggesting that degradation of granule proteins may be occurring in the absence of CgA in these transgenic mice. Additional *ex vivo* and *in vivo* studies will unveil the regulatory mechanism of DCG biogenesis mediated by CgA in adrenal medulla.

CgA deficiency and DCG swelling: implication in CA storage

Depletion of CgA from the adrenal chromaffin cells in the CgA–AS transgenic animals changed not only the number of DCGs formed but also the size of DCGs in these cells. The concentration of soluble cargo molecules such as CAs and ATP seems to be a determinant for the diameter of DCGs (Warashina, 1985; Colliver et al., 2000; Gong et al., 2003). When the intragranular concentration of CAs was challenged in PC12 cells and primary chromaffin cells by adding its precursor, L-3,4-dihydroxyphenyalanine (L-DOPA), DCGs swelled significantly. The swelling of DCGs with L-DOPA treatment was interpreted as a means of maintaining a constant concentration of "free" CAs (unbound to CgA) in the lumen of these granules (Colliver et al., 2000; Gong et al., 2003). In addition, the augmented concentration of ATP, another cargo abundant in DCGs, also increased the size of the granules (Warashina, 1985). Therefore, the total concentration of cargo molecules including CAs and ATP affects the morphology of DCGs in adrenal chromaffin cells. Because CA binds to CgA saturably (Videen et al., 1992), it is expected that the overall level of CA binding to CgA is limited because of the decrease of CgA in CgA–AS transgenic mice. If DCGs formed in CgA–AS transgenic mice are quantitatively less, but qualitatively comparable with WT DCGs, and CA biosynthesis remains unchanged, the amount of CAs per DCG would be expected to increase. This would result in an increase of free CAs in DCGs and accounts for the swelling of the DCGs in CgA–AS transgenic mice, similar to that observed in PC12 cells and adrenal chromaffin cells treated with L-DOPA (Colliver et al., 2000; Gong et al., 2003). Increase in DCG diameter and the area occupied by DCGs within the cells in the transgenic mice were well correlated with the amount of CgA reduction. With ~32% reduction in CgA, the average DCG diameter was increased to ~20%, and the total DCG area within the cells was increased to ~33% in TG-4. With ~69% reduction in CgA in TG-5, the average DCG diameter and their area within the cells were increased to ~38 and ~48%, respectively. Analysis on dense-core size showed only a slight, but statistically significant decrease (~4–7%) in the transgenic mice (Fig. 3*B*). This small reduction may possibly be associated with a technical limitation in the measurement of dense-core size in the enlarged DCGs (especially those with a diameter >400 nm) present mostly in the transgenic mice. Single sections of such large DCGs for EM would have less of a chance to represent a plain of section exactly in the middle of cores, giving rise to an apparent smaller core size. Such a small change in core size in the transgenic mice would suggest that the CgA level per DCG is not very different from the control mice and would have little contribution to the swelling of DCGs. Decrease in DCG numbers, enlargement in DCG size, and only a slight decrease in core size in the transgenic mice support our proposal that the CA levels in the chromaffin cells did not change but is accommodated as free CA in fewer DCGs by increasing DCG volume, because the binding of CA to CgA may be saturated.

In summary, the use of CgA–AS transgenic animals demonstrated the importance of CgA in dictating the number of DCG formed in a dose-dependent manner *in vivo*. Furthermore, deficiency in CgA in DCGs resulted in an increase in their size, presumably to maintain a constant concentration of free CA in the lumen of DCGs.

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.

Arnaoutova I, Smith AM, Coates LC, Sharpe JC, Dhanvantari S, Snell CR, Birch NP, Loh YP (2003) The prohormone processing enzyme PC3 is a lipid raft-associated transmembrane protein. Biochemistry (Mosc) 42:10445–10455.

Beuret N, Stettler H, Renold A, Rutishauser J, Spiess M (2004) Expression of regulated secretory proteins is sufficient to generate granule-like structures in constitutively secreting cells. J Biol Chem 279:20242–20249.

Chanat E, Huttner WB (1991) Milieu-induced, selective aggregation of regulated secretory proteins in the trans-Golgi network. J Cell Biol 115:1505–1519.

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

Colomer V, Kicska GA, Rindler MJ (1996) Secretory granule content proteins and the luminal domains of granule membrane proteins aggregate in vitro at mildly acidic pH. J Biol Chem 271:48–55.

Cool DR, Normant E, Shen F, Chen HC, Pannell L, Zhang Y, Loh YP (1997) Carboxypeptidase E is a regulated secretory pathway sorting receptor: genetic obliteration leads to endocrine disorders in Cpe(fat) mice. Cell 88:73–83.

Eisenhofer G, Kopin IJ, Goldstein DS (2004) Catecholamine metabolism: a contemporary view with implications for physiology and medicine. Pharmacol Rev 56:331–349.

Erickson JD, Eiden LE, Hoffman BJ (1992) Expression cloning of a reserpine-sensitive vesicular monoamine transporter. Proc Natl Acad Sci USA 89:10993–10997.

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.

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

Kim T, Tao-Cheng JH, Eiden LE, Loh YP (2003) The role of chromogranin A and the control of secretory granule genesis and maturation. Trends Endocrinol Metab 14:56–57.

Knoch KP, Bergert H, Borgonovo B, Saeger HD, Altkruger A, Verkade P, Solimena M (2004) Polypyrimidine tract-binding protein promotes insulin secretory granule biogenesis. Nat Cell Biol 6:207–214.

Liu XD, Sun ZQ, Zhang CB, Wu HL (2001) Construction of CgA gene antisense transgenic mice. Yi Chuan Xue Bao 28:493–501.

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.

Warashina A (1985) Changes in the size of isolated chromaffin granules in ATP-evoked catecholamine release. FEBS Lett 184:87–89.

Westermann R, Stogbauer F, Unsicker K, Lietzke R (1988) Calcium-dependence of chromogranin A-catecholamine interaction. FEBS Lett 239:203–206.

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