A Functional Interaction between Chromogranin B and the Inositol 1,4,5-Trisphosphate Receptor/Ca\(^{2+}\) Channel*

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Chromogranins A and B (CGA and CGB) are high-capacity, low affinity calcium (Ca\(^{2+}\)) storage proteins found in many cell types most often associated with secretory granules of secretory cells but also with the endoplasmic reticulum (ER) lumen of these cells. Both CGA and CGB associate with inositol 1,4,5-trisphosphate receptor (InsP\(_3\)R) in a pH-dependent manner. At an intraluminal pH of 5.5, as found in secretory vesicles, both CGA and CGB bind to the InsP\(_3\)R. When the intraluminal pH is 7.5, as found in the ER, CGA totally dissociates from InsP\(_3\)R, whereas CGB only partially dissociates. To investigate the functional consequences of the interaction between the InsP\(_3\)R and CGB monomers or CGA/CGB heteromers, purified mouse InsP\(_3\)R type I were fused to planar lipid bilayers and activated by 2 \(\mu\)M InsP\(_3\). In the presence of luminal CGB monomers or CGA/CGB heteromers the InsP\(_3\)R/Ca\(^{2+}\) channel open probability and mean open time increased significantly. The channel activity remained elevated when the pH was changed to 7.5, a reflection of CGB binding to the InsP\(_3\)R even at pH 7.5. These results suggest that CGB may play an important modulatory role in the control of Ca\(^{2+}\) release from the ER. Furthermore, the difference in the ability of CGA and CGB to regulate the InsP\(_3\)R/Ca\(^{2+}\) channel and the variability of CGA/CGB ratios could influence the pattern of InsP\(_3\)-mediated Ca\(^{2+}\) release.

Chromogranin B (CGB)\(^1\) belongs to the granin protein family, which also includes chromogranin A (CGA) and secretogranin II (chromogranin C). It is found in the large dense core secretory granules and in the endoplasmic reticulum (ER) lumen of most neurons, exo/endocrine cells, and neuroendocrine cells and shows a wide distribution in various areas of the brain (1). Levels of CGB expression in cells can be used as markers for a number of physiological and medically important pathophysiological conditions (2–4). In normal brain tissue CGB expression is enhanced after neuronal activation, providing a marker for stimulated neurons (3). In addition to the tissue-specific distribution, a regionally specific distribution of CGB has been found intracellularly in neurally differentiated pheochromocytoma (PC12) cells (5). In these cells CGB levels are elevated in the neurites rather than in the soma, which correlates with the initiation site for intracellular calcium (Ca\(^{2+}\)) signals. The levels of CGB and chromogranin-derived peptides can be diagnostic markers for pathophysiological conditions. For example, levels of CGB are greatly reduced in the cerebrospinal fluid of chronic schizophrenic subjects (6, 7). Moreover, the levels of CGB and chromogranin-derived peptides are diagnostically significant as neuronal markers for synaptic degeneration in Alzheimer's disease (4).

At the cellular level CGB is believed to have many intra- and extracellular functions. CGB functions as a heparin binding extracellular matrix protein, mediating adhesion of cells and supporting neurite outgrowth (8). CGB is a prohormone with numerous di- and tribasic amino acid cleavage sites that act as targets for proteolytic enzymes such as the prohormone convertase (9, 10). Furthermore, chromogranin B is known to bind >90 mol of Ca\(^{2+}\)/mol with a dissociation constant \((K_d)\) of 1.5 mM (11), distinguishing itself as a very efficient Ca\(^{2+}\) storage protein. Intracellularly, CGB has also been suggested to participate in packaging and sorting other proteins into the secretory vesicles of neuroendocrine cells, thus playing key roles in secretory granule biogenesis (12–14). Indeed, CGB has recently been shown to induce secretory granule biogenesis (15). CGB also localizes to the nucleus and controls transcription of many genes, including those for transcription factors (16).

In secretory granules both CGA and CGB have been shown to interact with the InsP\(_3\)R at the intravesicular pH of 5.5 (11, 17). Purified InsP\(_3\)R interacts directly with CGA and CGB at pH 5.5. CGB dissociates from the InsP\(_3\)R at pH 7.5, whereas CGB remains partially associated (18). Both chromogranin proteins form a complex with the InsP\(_3\)R in vivo (11). The functional aspect of this coupling has been investigated for CGA alone using single channel experiments and Ca\(^{2+}\) flux studies (19). In the presence of CGB the open probability and mean open time of the InsP\(_3\)R channel increases 10-fold.

Despite the role of secretory granules of secretory cells and ER as major InsP\(_3\)-sensitive intracellular Ca\(^{2+}\) stores (13, 20, 21) and the abundance of CGB in these (10) and a variety of other cell types (1), the functional interaction of InsP\(_3\)R and CGB is less well characterized. Given also the tendency of CGA/CGB mixture to spontaneously form a CGA/CGB heterodimer at a near physiological pH 7.5 and a Ca\(_{\text{stab}}\) of the InsP\(_3\)R/Ca\(^{2+}\) channel increases 10-fold.

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heterotetramer at the intragranular pH 5.5 (22), it is important to understand the effects of CGB and CGA/CGB heteromers. In this study, we examined the effect of CGB and CGA/CGB heteromers on the channel gating properties of the InsP$_3$R type I. CGB increased the open probability and mean open time of the channel by almost 20-fold. However, in contrast to CGA, this functional effect was less sensitive to changes in pH when compared with the effect of CGA alone. These results show the functional interaction of InsP$_3$R with CGB monomers and CGA/CGB heteromers and suggest that regulation of these interactions plays a physiologically important role in determining the pattern of InsP$_3$-mediated Ca$^{2+}$/H$^{+}$ release.

MATERIALS AND METHODS

Antibody—An InsP$_3$R peptide specific to terminal 10–13 amino acids of type 1 (HPPHMNVNPQQPA) was synthesized with a carboxyl-ter-

FIG. 1. pH-dependent interaction of InsP$_3$R type I with CGA, CGB, and CGA/CGB. Purified InsP$_3$R type I (0.5–0.7 µg) was reacted with the GST fusion proteins of CGA, CGB, and an equimolar mixture of GST-CGA and GST-CGB at both pH 5.5 and 7.5. The bound InsP$_3$R type I was separated on 7.5% SDS-gels and analyzed by immunoblot using a type 1-specific InsP$_3$R antibody specific for the type I isoforms (11).

FIG. 2. Effects of CGB and CGA/CGB on InsP$_3$-induced Ca$^{2+}$ release from InsP$_3$R-reconstituted liposomes. InsP$_3$-induced Ca$^{2+}$ efflux through the proteoliposomes (300 µM Ca$^{2+}$ inside) was determined by the fluorescence change of 10 µM indo-I at 393 nm after a series of incremental additions of InsP$_3$ (2.0 µM final) to the proteoliposome solution. A, the InsP$_3$-induced Ca$^{2+}$ release was measured both in the presence of encapsulated CGB at intraliposomal pH of 5.5 (●) and 7.5 (○) and in the absence at pH 5.5 (■) and 7.5 (□). The figure shows the amount of released Ca$^{2+}$ expressed as percentage of maximum releasable Ca$^{2+}$. InsP$_3$-induced fluorescent changes were compared with that obtained by the addition of 1% Triton X-100 (this value was set at 100%). B, the InsP$_3$-induced Ca$^{2+}$ release was measured in the presence of an equimolar mixture of CGA and CGB at an intraliposomal pH of 5.5 (●) and 7.5 (○) and in the absence at pH 5.5 (■) and 7.5 (□). The figure shows the amount of released Ca$^{2+}$ expressed as percentage of maximum releasable Ca$^{2+}$. InsP$_3$-induced fluorescent changes were compared with that obtained by the addition of 1% Triton X-100 (this value was set at 100%).

FIG. 3. Quenching of InsP$_3$R fluorescence by iodide in the presence of CGB and CGA/CGB. A, the changes in tryptophan fluorescence from the proteoliposomes containing InsP$_3$R only at pH 5.5 (●) or pH 7.5 (□) or InsP$_3$R and CGB either at pH 5.5 (●) or pH 7.5 (○) were measured as a function of increasing concentrations of KI. $F_o$ and $F$ represent the fluorescence intensities in the presence ($F$) and absence ($F_o$) of KI. $B$, the changes in tryptophan fluorescence from the proteoliposomes containing InsP$_3$R only at pH 5.5 (●) or pH 7.5 (□) or InsP$_3$R and CGA/CGB mixture either at pH 5.5 (●) or pH 7.5 (□) were measured as a function of increasing concentrations of KI. $F_o$ and $F$ represent the fluorescence intensities in the presence ($F$) and absence ($F_o$) of KI.
minal cysteine, and anti-rabbit polyclonal antibody was raised. The polyclonal anti-rabbit antibody was affinity-purified on the immobilized peptide following the procedure described (23), and the specificity of the antibody was confirmed (11).

**InsP3R Interaction with Glutathione S-Transferase (GST)-CGA/CGB**

To construct GST fusion proteins, bovine CGA cDNA (24) and CGB cDNA (25) were amplified by PCR and subcloned into vector pGEX-5T (Amersham Biosciences). GST-CGA and GST-CGB fusion proteins were expressed in *Escherichia coli* BL21 cells and purified on glutathione-agarose beads. The binding reactions were carried out either in a pH 5.5 buffer (20 mM sodium acetate, pH 5.5, 0.1 M KCl, 2 mM CaCl₂, and 0.1% Triton X-100) or in a pH 7.5 buffer (20 mM Tris-HCl, pH 7.5, 0.1 M KCl, 2 mM CaCl₂, and 0.1% Triton X-100), and the interaction was determined by incubating an excess amount of purified GST-CGA or -CGB fusion protein (20 μg) with the purified InsP₃R (0.5–0.7 μg) in 0.5 ml of buffer supplemented with 1× protease inhibitor mixture (Roche Applied Science) for 1 h at 4 °C. After incubation the reaction mixture was rinsed with 0.5 ml of buffer to remove unbound proteins. Elution of the bound InsP₃R was carried out by using three bed volumes of the pH 7.5 buffer but with 1 M KCl. The eluted InsP₃R was resolved on a 7.5% SDS-polyacrylamide gel and identified by chemiluminescence-based immunoblot analysis using the affinity-purified InsP₃R antibody.

**Purification of the InsP₃R**—For flux studies the InsP₃R type I was isolated from bovine cerebella as described previously (26). Briefly, bovine cerebella were mixed with 3 volumes of buffer I (50 mM Tris-HCl, pH 7.4, 0.32 M sucrose, 1 mM EDTA, 1 mM DTT, 0.1 mM phenylmethylsulfonyl fluoride, 10 μM leupeptin, 10 μM pepstatin), homogenized, and centrifuged at 2000 g for 10 min at 4 °C. The supernatants were re-centrifuged at 105000 g for 1 h to precipitate the membrane pellet, which was resuspended in buffer II (50 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM DTT, 0.1 mM phenylmethylsulfonyl fluoride, 10 μM leupeptin, 10 μM pepstatin) containing 1% Triton X-100, stirred for 1 h, and then centrifuged at 32000 × g for 1 h at 4 °C. The resulting supernatant was mixed with an equal volume of buffer III (20 mM Tris-HCl, pH 7.5, 0.1 M NaCl, 1% Triton X-100, 1 mM β-mercaptoethanol, 0.1 mM phenylmethylsulfonyl fluoride, 10 μM leupeptin, 10 μM pepstatin) and applied to an InsP₃R antibody-coupled

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**Fig. 4. Single channel activity and mean open time in the presence and absence of CGB.**

A, i and ii, InsP₃R single channels before and after activation by 2 μM InsP₃ (added to the cis compartment). Openings are defined as downward deflections from the base line. iii, conditions are the same as for ii, except CGB (1 μg) was added to the trans compartment and mixed. The pH of the trans compartment was pH 5.5. iv, the pH in the trans compartment was changed by the addition of Tris (final concentration 55 mM) to pH 6.5. v, the trans pH was further changed by the addition of Tris (55 mM) to give a pH of 7.5. vi, the addition of heparin inhibited all channel activity. B, top panel, mean open times for InsP₃R in the absence of CGB. One population of open times was observed with a value of 5.2 ± 1.5 ms. Middle panel, mean open times for InsP₃R in the presence of CGB at trans pH 5.5. Two populations of open times were observed with values of 3.1 ± 0.3 and 217.4 ± 0.3 ms. Bottom panel, mean open times for InsP₃R after partial dissociation of CGB by pH change (trans pH 7.5). Two populations of open times were observed with values of 4.8 ± 0.6 and 122.5 ± 0.7 ms. The open times were less than at pH 5.5, but had not returned to control levels. This experiment is typical of four similar but separate experiments.
**RESULTS**

**pH-dependent Interaction of InsP$_3$R with CGA, CGB, and CGA/CGB**—To determine whether there was a direct interaction between the purified InsP$_3$R and CGA or CGB, GST fusion forms of CGA and CGB were expressed in *E. coli* BL21 and purified. The interaction between the InsP$_3$R and GST-CGA and -CGB fusion proteins was examined at pH 5.5 and 7.5 (Fig. 1). As shown in Fig. 1 (left side of the figure), CGA, CGB, and a mixture of CGA and CGB (CGA/CGB) all interacted with the InsP$_3$R at pH 5.5. When tested at pH 7.5 CGA failed to interact with the InsP$_3$R (Fig. 1, right side), but CGB still interacted with the InsP$_3$R, albeit at a reduced level (Fig. 1, right side), reflecting a stronger affinity of CGB for the InsP$_3$R.

**Effects of CGB and CGA/CGB on InsP$_3$-mediated Ca$^{2+}$ Release**—Ca$^{2+}$ release studies were employed to investigate the effects of CGB on the InsP$_3$ concentration-response for InsP$_3$R type I. As previously described for CGA (19), InsP$_3$-induced Ca$^{2+}$ release from InsP$_3$R-reconstituted liposomes was monitored at two different pH values both in the presence and absence of CGB (Fig. 2A). InsP$_3$-induced Ca$^{2+}$ efflux was determined using proteoliposomes containing 300 μM Ca$^{2+}$. The total amount of InsP$_3$-releasable Ca$^{2+}$ was estimated to be 62–70%. The InsP$_3$-induced Ca$^{2+}$ release obtained in the absence of CGB gave a $K_{app}$ value for InsP$_3$ of 0.65 μM. When CGB was present inside the vesicle at pH 5.5, the pH value at which CGA and CGB coupled with the InsP$_3$R (18), InsP$_3$-induced Ca$^{2+}$ release was markedly enhanced (see Fig. 2A), yielding a $K_{app}$ value for InsP$_3$ of 0.16 μM. Interestingly, when the pH was maintained at 7.5, the fluxes measured at each InsP$_3$ concent-

### Table I

| Treatment | pH 5.5 | pH 5.5 | pH 7.5 |
|-----------|--------|--------|--------|
| Control   | 0.5    | 3.3    | 0.07   |
| +CGA      | 71.7   | 0.8    | 10.0   |
| +CGB      | 5.2    | 3.1    | 4.8    |
| +CGB+CGB  | 217.4  | 0.3    | 122.5  |

### Table II

| Treatment | pH 5.5 | pH 5.5 | pH 7.5 |
|-----------|--------|--------|--------|
| Control   | 4.0    | 33.0   | 3.0    |
| +CGA      | 80.0   | 9.0    | 4.0    |
| +CGB+CGB  | 3.0    | 77.0   | 48.0   |

**Fluorescence intensity at the emission wavelength of 340 nm was measured with the fluorescence spectrophotometer equipped with a temperature-controlled cuvette holder. Fluorescence intensity was measured at the emission wavelength of 380 nm with excitation of 355 nm on the right side figure.**
tration were similar to those seen at pH 5.5 in the presence of CGB (K_{app} value for InsP3 of 0.17 M). This result is distinct from that obtained with CGA where pH had a profound effect, and the response at pH 7.5 was similar to that measured in the absence of CGA (19).

Ca^{2+} release from the InsP3R-reconstituted liposomes was also monitored in the presence of an equimolar mixture of CGA/CGB (Fig. 2B). The K_{app} value for InsP3 was approximately the same in the presence of the mixtures as that measured in the presence of CGB alone (K_{app} value for InsP3 was 0.15 μM at pH 5.5 and 0.18 μM at pH 7.5).

Changes in InsP3R Conformation—To investigate possible conformational changes of the InsP3R by its interaction with monomeric CGB or a heteromeric CGA/CGB, we utilized collisional quenching of the intrinsic tryptophan (Trp) fluorescence of the InsP3R by iodide (Fig. 3). By determining the extent of quenching, it was possible to determine whether the Trp environment of the InsP3R is changed as a result of a change in the InsP3R conformation. As shown in Fig. 3, the InsP3R Trp fluorescence was quenched by iodide in the presence of both CGB or the CGA/CGB mixture. The emission fluorescence at 340 nm was measured, and the results were plotted according to the Stern-Volmer equation (29), F_0 - F = K_{sv}[I], where F_0 is the emission intensity in the absence of iodide, F is the intensity in the presence of iodide, K_{sv} is the Stern-Volmer quenching constant, and [I] is the molar concentration of iodide. The K_{sv} value estimated from the slope was 2.94 M^{-1} for the reconstituted InsP3R in the absence of CGB. When CGB was present, this value decreased to 2.51 and 1.55 M^{-1} at the intraliposomal pH 7.5 and 5.5, respectively (Fig. 3A). From this experiment it is clear that at least some Trp residues of the InsP3R are less exposed to the solvent when CGB is present, demonstrating the CGB-induced conformational changes of the InsP3R. Furthermore, the K_{sv} values suggest that the Trp residues are less exposed when the intraliposomal pH was maintained at 5.5 than at 7.5.

There also were conformational changes of the InsP3R in the presence of a CGA/CGB mixture in the liposome (Fig. 3B). The K_{sv} value of 3.11 in the absence of CGA/CGB was decreased to 2.25 and 2.06 M^{-1} at the intraliposomal pH 7.5 and 5.5, respectively (Fig. 3A). From this experiment it is clear that at least some Trp residues of the InsP3R are less exposed to the solvent when CGB is present, demonstrating the CGB-induced conformational changes of the InsP3R. Furthermore, the K_{sv} values suggest that the Trp residues are less exposed when the intraliposomal pH was maintained at 5.5 than at 7.5.

Effects of CGB and CGA/CGB on InsP3R Channel Activity—The effects of CGB and CGA/CGB on InsP3R function were investigated at the single channel level using InsP3R incorporated into planar lipid bilayers. In the absence of luminal CGB and in the presence of cytosolic-free Ca^{2+} (300 nM) and InsP3 (2 μM) InsP3R single channel activity was observed (see Fig. 4A, trace iii). Single channel currents of ~2 nA were seen, and a
single population of mean open times was obtained with a value that ranged between 5.2 and 8.6 ms (Fig. 4B, top panel; Table I). The open probability ($P_o$) was 5% ($n = 4$; Table II).

After the addition of 1 μg of CGB to the luminal compartment a dramatic increase in channel activity was observed (Fig. 4A, trace ii). Although the size of the single channel current remained unaltered, significant differences in mean open times and $P_o$ were seen. Two populations of mean open time were apparent, but the values were noticeably increased over control values (Fig. 4B, middle panel). Although the short open time remained in the same range as control values, a new, longer open time was evident that was at least 10-times larger than the open time observed under control conditions (3.1 ± 0.3 and 217.4 ± 0.3 ms are the open times in the presence of CGB; Table I). Furthermore, the $P_o$ increased from 5% in control conditions to 80% ($n = 4$; Table II) in the presence of CGB.

After changing the pH of the luminal compartment to pH 6.5, a condition causing partial dissociation of CGB from the InsP$_3$R, the $P_o$ was reduced to 63 ± 2%. Although this is a decrease in channel activity, the $P_o$ was still elevated when compared with control levels (Fig. 4A, trace iv). A further change in luminal pH to 7.5 (Fig. 4A, trace v) caused the $P_o$ to fall even further, but the value of 40% ± 2% still exceeded control levels, indicating that CGB remained coupled to the InsP$_3$R. The mean open times were reduced to 4.8 ± 0.6 and 122 ± 0.7 ms ($n = 4$; Fig. 4B, bottom panel and Table I). Addition of heparin, an InsP$_3$R-specific antagonist, to the cytoplasmic compartment inhibited channel activity completely (Fig. 4A, trace vi).

In the next series of experiments a mixture of CGA/CGB was tested. In the absence of CGA/CGB the amplitude of the single channel currents was 2 pA (see Fig. 5A, trace ii), the $P_o$ was 3.0 ± 1.0% ($n = 4$), and the mean open time was similar to that obtained in the previous control experiments (7.2 ± 0.2 ms). After the addition of 1 μg of CGA/CGB to the luminal compartment (pH 5.5) a large increase in channel activity was observed.
(Fig. 5A, trace iii). The Po was 77% ± 3% (n = 4), and two populations of mean open time were evident (11.1 ± 0.4 and 84.4 ± 0.3 ms; Table I). Although the values for the open times were increased over control values (Fig. 5B, top panel) the longer population of mean open times was less than that seen with CGB alone and more closely resembled that seen with CGA alone.

A subsequent change in the luminal pH to 7.5 (Fig. 5A, trace iv) caused Po to fall, but the value of 48 ± 2% again exceeded control levels. The elevated Po indicates a continued coupling of CGA/CGB with the InsP3R, potentially via the CGB component of the heteromer. The mean open times were 7.3 ± 0.3 and 120.9 ± 0.3 ms, values closer to CGB alone at pH 7.5 (n = 4; Fig. 5B, bottom panel; Table I). The addition of heparin to the cytoplasmic compartment inhibited channel activity completely (Fig. 5A, trace v).

As with previous studies demonstrating the effect of CGA on the activity of the InsP3R (19), several control experiments were done. 1) The addition of CGB or CGA/CGB in the absence of cytosolic InsP3 did not potentiate any InsP3R channel activity; 2) the addition of CGA or CGB/CGB to the luminal compartment in the absence of InsP3R had no effect upon the bilayer currents; 3) the addition of CGB or CGA/CGB to the cytoplasmic compartment in the presence of InsP3R and InsP3 did not affect channel activity.

**Effect of CGB or CGA/CGB on the InsP3 Concentration-Response for InsP3R.**—Single channel activity was observed over a range of InsP3 concentrations both in the presence of CGB at pH 5.5 and 7.5 and in its absence (Fig. 6A). Starting at an InsP3 concentration of 0.2 μM, the Po was greater in the presence of CGB (luminal pH 5.5) when compared with control levels, with an ~18-fold increase in Po at 2 μM InsP3 (Fig. 6A, middle panel). At pH 7.5 (Fig. 6A, right panel) the channel again has a higher Po at the lower InsP3 concentration range, although at 2 μM InsP3 the Po is less than that observed at pH 5.5.

Similar experiments to examine the InsP3 concentration dependence were done in the presence of the CGA/CGB heteromer (Fig. 6B). Again, in the presence of CGA/CGB, over a range of InsP3 concentrations starting at 0.2 μM, the open probability was greater in the presence of CGA/CGB (luminal pH 5.5) when compared with control levels (Fig. 6B, middle panel). Repetition of the experiment at pH 7.5 (Fig. 6B, right panel) indicated that the Po at the lower InsP3 concentrations were similar to those seen for CGA/CGB at pH 5.5, but at 2 μM InsP3 the Po was less at pH 7.5 than that seen at pH 5.5.

The InsP3 concentration dependence of channel activity after the addition of either CGB or the mixture of CGB/CGB was compared at pH 5.5 and 7.5 (Fig. 7). Regardless of the pH used the effect of CGB alone or CGA/CGB was similar (Fig. 7), suggesting that the properties of CGB, especially the ability to bind to the InsP3R at pH 7.5, predominates.

**DISCUSSION**

In the present study we examined the functional interaction between the InsP3R type I and CGB monomers or CGB/CGB heteromers and found that both CGB and CGB/CGB heteromers enhanced activation of the InsP3R/Ca2+ channel in the presence of InsP3. A direct physical interaction between the InsP3R and CGB or CGA was demonstrated (17), and the molecules co-localized in intact cells (5). Furthermore, a mixture of CGB/CGB interacted with the purified InsP3R at both pH 5.5 and 7.5, although the interaction at pH 7.5 was markedly reduced compared with that at pH 5.5 (Fig. 1). The stronger interaction between the InsP3R and the chromogranins at acidic pH is consistent with the physiological roles of secretory granules because the intragranular pH of secretory granules decreases and the Ca2+ content increases as the granules move from the trans-Golgi network to the plasma membrane.

The interaction of the InsP3R with CGB monomers and with CGA/CGB heterotetramers at pH 5.5 elicits an open probability for the InsP3R-gated Ca2+ channel of 80 and 77%, respectively, which is the highest activity of the InsP3R observed to date in a bilayer. This functional interaction at pH 5.5 is likely related to the association of these proteins in secretory granules (18). An elevation of intracellular Ca2+ is necessary for the process of fusion and exocytosis of secretory vesicles, and it is likely that the InsP3R/CGB interaction assists in this process. As the vesicles mature, the intraluminal pH becomes more acidic, and the Ca2+ is elevated (30). The mature vesicles accumulate near the plasma membrane, and exocytosis is more likely to occur for more acidic secretory granules (30). The increased association between CGB and the InsP3R as the vesicles mature will enhance the probability of exocytosis because the protein complex shows an increased sensitivity of the InsP3R to activation by InsP3. This enhanced channel activity will quickly release Ca2+ into the microregion between the vesicle and the plasma membrane where the fusion apparatus awaits the Ca2+ required for exocytosis.

The functional interaction between the InsP3R and CGB
remains even when the pH is at a near physiological level, suggesting that this protein association has roles in numerous locations in a cell. The addition of CGB or CGA/CGB heteromers to the InsP₃R at pH 7.5 elicited an open probability of 40 and 48%, respectively. Again, the level of activation at this pH was elevated from those reported previously when using isolated InsP₃R (31, 32). The association between the InsP₃R and CGB at pH 7.5 can have important regulatory roles in the ER, where CGB is found intraluminally and the InsP₃R is associated with the ER membrane. Although the distribution of the InsP₃R is assumed to be relatively homogeneous, CGB has been shown to be distributed in a regionally specific manner, as seen in neuronally differentiated PC12 cells (5). In these cells CGB is preferentially localized to the neurites, which is the region where intracellular Ca²⁺ signals initiate (5). It is likely that this scenario will be found in other cell types as well, with the association of CGB with the InsP₃R being important in modulating the time needed to achieve maturity in different Ca²⁺ release types both from secretory granules and from the ER.

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