The Journal of Biological Chemistry Vol. 279, No. 34, Issue of August 20, pp. 35551–35556, 2004
© 2004 by The American Society for Biochemistry and Molecular Biology, Inc.
Printed in U.S.A.

Functional Coupling of Chromogranin with the Inositol 1,4,5-Trisphosphate Receptor Shapes Calcium Signaling*

Received for publication, October 13, 2003, and in revised form, June 8, 2004
Published, JBC Papers in Press, June 11, 2004, DOI 10.1074/jbc.M311261200

Chi-Un Choe‡§, Kenneth D. Harrison‡, Wayne Grant‡, and Barbara E. Ehrlich‡¶

From the ‡Department of Pharmacology and Cellular & Molecular Physiology, Yale University, New Haven, Connecticut 06520 and §Institute for Neural Signal Transduction, Zentrum fuer Molekulare Neurobiologie Hamburg, University of Hamburg, 20251 Hamburg, Germany

Chromogranins A and B are high capacity, low affinity calcium (Ca^{2+}) storage proteins that bind to the inositol 1,4,5-trisphosphate-gated receptor ($InsP_3R$). Although most commonly associated with secretory granules of neuroendocrine cells, chromogranins have also been found in the lumen of the endoplasmic reticulum (ER) of many cell types. To investigate the functional consequences of the interaction between the $InsP_3R$ and the chromogranins, we disrupted the interaction between the two proteins by adding a chromogranin fragment, which competed with chromogranin for its binding site on the $InsP_3R$. Responses were monitored at the single channel level and in intact cells. When using $InsP_3R$ type I incorporated into planar lipid bilayers and activated by cytoplasmic $InsP_3$ and luminal chromogranin, the addition of the fragment reversed the enhancing effect of chromogranin. Moreover, the expression of the fragment in the ER of neuronally differentiated PC12 cells attenuated agonist-induced intracellular Ca^{2+} signaling. These results show that the $InsP_3R$/chromogranin interaction amplifies Ca^{2+} release from the ER and that chromogranin is an essential component of this intracellular channel complex.

Ca^{2+} functions as a second messenger in excitable and nonexcitable cells, playing an important role in development, gene transcription, synaptic modulation, and secretion (1, 2). The cytosolic Ca^{2+} concentration can be increased by entry of extracellular Ca^{2+} via voltage-operated, receptor-operated, or store-operated Ca^{2+} channels (3). In addition, Ca^{2+} release from intracellular stores by ryanodine receptors and inositol 1,4,5-trisphosphate receptors ($InsP_3R$)[1] plays a pivotal role in the modulation of intracellular Ca^{2+} signals (1, 4–6).

The $InsP_3R$ type 1, which is predominantly found in the cerebellum, plays an important role in neuronal functions such as long term potentiation and depression (7) and has recently been implicated in the etiology of neurological diseases such as Huntington's disease (8). The $InsP_3R$ is regulated by phosphorylation (9), ubiquitation (10), and association with luminal and cytosolic proteins (11, 12). Among the proteins associating with the $InsP_3R$, only the high capacity, low affinity Ca^{2+} storage proteins, chromogranins A and B (CGA and CGB), have been shown to bind to the $InsP_3R$ on the luminal side (13, 14). CGA and CGB are members of the granin family and can be found in a wide variety of endocrine and neuroendocrine cells as well as in neurons of the peripheral and central nervous systems (15, 16).

CGA and CGB have numerous extracellular and intracellular functions. One extracellular function is that chromogranins act as hormones after being proteolytically processed into peptides such as pancreastatin (17, 18), vasostatins I and II (19–21), parastatin (22), catestatin (23), and chromacin (24). Elevated levels of circulating chromogranins were found in patients with endocrine and neuroendocrine tumors, renal failure, and heart failure. Chromogranins and their metabolites were also found secreted into the cerebrospinal fluid in patients with neurological disorders.

Intracellular roles for the chromogranins include the control of dense-core secretory granule formation, protein sorting, and Ca^{2+} storage. These functions may prove to be essential to neurons as the expression levels of chromogranin are altered in patients with Pick's disease, Alzheimer's disease, Parkinson's disease, and schizophrenia (25). Recently the interaction of the $InsP_3R$ with CGA, CGB, or chromogranin A and B heteromer (CGAB) was shown to be functional using single channel and ion flux studies (12, 26). The molecular interaction of the $InsP_3R$ with CGB requires a 20 amino acid sequence near the N-terminal region of CGB (CGB fragment) and the L3-2 intraluminal loop of $InsP_3R$ (27).

In this study, we show that the CGB fragment can inhibit the functional interaction between CGAB and the $InsP_3R$ in single channel measurements. Moreover, we demonstrate that the binding of the CGB fragment alone is not sufficient to elicit the functional effect of full-length chromogranin. We also show that the functional interaction between CGAB and the $InsP_3R$ can be disrupted *in vivo*. Specifically, the expression of the CGB fragment in differentiated PC12 cells inhibits intracellular Ca^{2+} signaling, showing that the CGAB/$InsP_3R$ interaction amplifies Ca^{2+} signaling in this established neuron model (28). The ability to regulate the CGAB/$InsP_3R$ interaction will have important consequences for intracellular Ca^{2+} levels in all cells where these proteins are found.

EXPERIMENTAL PROCEDURES

Plasmids—The cDNA encoding for the conserved near N-terminal CGB peptide with the sequence IIEVLSNALLKSSAPPITPE (residues 17–36) and the cDNA encoding for a scrambled peptide with the sequence LAPEPIEKLPTSAVILNSSI (same amino acids as the CGB fragment but randomized) were each subcloned into pShooter-pCMV/Myc/ER (Invitrogen). Both constructs were verified by restriction en-

* This work was supported by Grant GM63496 from the National Institutes of Health and a German National Merit Foundation scholarship (to C. C.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶ To whom correspondence should be addressed: Dept. of Pharmacology, 333 Cedar St., Yale University, New Haven, CT 06520-8066. Tel.: 203-737-1158; Fax: 203-785-7670; E-mail: barbara.ehrlich@yale.edu.

[1] The abbreviations used are: $InsP_3R$, inositol 1,4,5-trisphosphate receptors; CGA and CGB, chromogranins A and B; CGAB, chromogranin A and B heteromer; GFP, green fluorescent protein; NGF, nerve growth factor; PBS, phosphate-buffered saline; HPLC, high pressure liquid chromatography; ER, endoplasmic reticulum.

Downloaded from www.jbc.org at Yale University on December 15, 2006

zyme analysis and sequencing. A pcDNA3.1 DsRed2-N1 construct (Clontech) was co-transfected to verify transfection. For immunocytochemistry experiments, the pShooter-pCMV/GFP-Myc/ER vector (Invitrogen) was used.

Cell Culture and Transfection—PC12 cells were maintained and propagated as described previously (29).[2] For Ca^{2+} imaging and immunocytochemistry experiments, PC12 cells were plated on collagen and poly-L-lysine-coated coverslips (BD Biocoat) at a concentration of 3.5×10^4 cells/ml. 20–30 h after plating, cells were co-transfected with 0.5 μg of pcDNA3.1 DsRed2-N1 cDNA and 1.5 μg of pShooter-pCMV/Myc/ER containing either the CGB fragment or the scrambled peptide. After transfection, PC12 growth medium was supplemented with 100 ng/ml nerve growth factor (NGF, Alomone Laboratories). Cells were used after differentiation for 6 days and a confluency of 20%.

Western Blot and Immunofluorescence—CGA and CGB were detected using standard Western blotting techniques (Santa Cruz goat CGA antibody at a 1:100 dilution and Santa Cruz mouse CGB antibody at a 1:100 dilution).

After NGF differentiation for 6 days, cells were fixed with 2% paraformaldehyde in PBS for 20 min at room temperature. After washing with PBS, cells were permeabilized and blocked with 0.3% Triton X-100, 10% goat serum, and 1% bovine serum albumin in PBS for 1 h. Primary antibodies (mouse anti-Myc and rabbit anti-calnexin) were used at a 1:100 dilution in PBS supplemented with 1% goat serum for 1 h, and secondary antibodies (anti-mouse Alexa Fluor 488 and anti-rabbit Alexa Fluor 594) were used at a 1:500 dilution in PBS supplemented with 1% goat serum for 1 h. Samples were examined using laser confocal microscopy (Zeiss LSM510 Meta).

Ca^{2+} Imaging—Confocal microscopy was used to measure intracellular Ca^{2+} in differentiated PC12 cells as described previously (29).[2] L15 medium was used as the extracellular solution. Most experiments were performed using extracellular medium containing 5 mM EGTA to deplete extracellular calcium. When comparisons were made between cells incubated with or without extracellular calcium, essentially the same result was obtained. After loading the PC12 cells with Fluo-4 AM, the dye was excited at 488 nm and the emission signal was detected at 522–535 nm. Images were recorded at a rate of 2–4 Hz at room temperature. Only fluorescent changes over somatic regions of differentiated and transfected (DsRed-positive) PC12 cells were analyzed. A 9:1 running average filter was applied to the representative traces shown (29).[2] The moving average filter is a simple low pass filter that can be imagined as a window of a nine-element size moving along the data array, one element at a time. The middle element of the window is replaced with the average of all of the elements in the window.

Purification of the $InsP_3R$—The mouse $InsP_3R$ type I was purified as described previously (31). The purified $InsP_3R$ was incorporated into liposomes by the addition of 100 μg of purified protein to 1 ml of liposome solution (consisting of phosphatidylcholine/phosphatidylserine (3:1) in bilayer buffer), mixing, and then incubating on ice for 10 min.

Synthesis of the CGB Fragment and the Scrambled Peptide—The CGB fragment and the scrambled peptide were synthesized by a small scale peptide synthesis using a Rainin Symphony instrument that provides on instrument cleavage of the peptide from the resin. Crude peptides were analyzed by reverse-phase HPLC profile and a matrix-assisted laser desorption ionization mass spectrum. Purified peptides were a minimum of 99% pure based on the accompanying absorbance profile from an analytical reverse-phase HPLC run performed on an aliquot of the purified pool.

Bilayer Experiments—$InsP_3R$ channel activity in planar bilayers was monitored and analyzed as described previously (12, 26). The two compartments are defined as *cis* (corresponding to the cytosol) and *trans* (corresponding to the ER lumen). After adding a mixture of CGA/CGB (1:1) to the luminal compartment, $InsP_3R$ channel activity was recorded at 0 mV in the presence of increasing concentrations of the CGB fragment or the scrambled peptide.

RESULTS

The CGB Fragment Inhibits the CGAB/$InsP_3R$ Functional Coupling in Vitro—The effect of the CGB fragment on the functional coupling of CGAB and $InsP_3R$ was investigated at the single channel level using purified $InsP_3R$ incorporated into planar lipid bilayers. Under control conditions, in the presence of 300 nM cytosolic-free Ca^{2+} and 2 μM $InsP_3$, $InsP_3R$ single channel activity was observed (Fig. 1*A*, *trace I*) with a current amplitude of ~2 pA and an open probability of 3.0 ± 0.7% ($n = 4$). After the addition of CGAB to the luminal compartment (10 nM of final concentration), a dramatic change in channel activity was observed (Fig. 1*A*, *trace II*). The magnitude of the single channel current was unaltered, but the open probability increased dramatically to 19 ± 2% ($n = 3$).

After activating the channel by the addition of $InsP_3$ to the cytoplasmic side and CGAB to the luminal side, the CGB fragment was added to the luminal chamber. Channel activity was recorded over a CGB fragment concentration range of 0.001–60 μM. Increasing the CGB fragment concentration up to 0.05 μM had no effect on the open probability (Fig. 1*A*, *trace III*), but further additions of the CGB fragment decreased the open probability. Maximal activity dropped to 3%, the value for the open probability before the addition of CGAB, over the concentration range of 0.1–6 μM (Fig. 1, *A*, *traces IV* and *V*, and *C*). Subsequent additions of the CGB fragment up to 60 μM had no further effect on the open probability (Fig. 1*A*, *trace VI*). The absolute open probability values were plotted as a function of the CGB fragment concentration, and an IC_{50} of ~470 nM CGB fragment was calculated (Fig. 1*C*; $n = 4$).

As a control, the effect of a scrambled peptide (the 20 amino acids of the CGB fragment but randomized) was compared with the effects of the CGB fragment. In the presence of 10 nM luminal CGAB, 300 nM cytosolic-free Ca^{2+}, and 2 μM cytosolic $InsP_3$ and in the absence of the scrambled peptide, the single channel activity was similar to that observed before the addition of CGB fragment (compare Fig. 1, *A* and *B*; an open probability of 21 ± 3%; $n = 3$). The addition of the scrambled peptide over the entire concentration range tested (0.05–30 μM) elicited no significant change in the open probability (Fig. 1, *B* and *D*). The effect of the CGB fragment on the activity of the $InsP_3R$ in the absence of CGAB was also tested. After the addition of 500 nM CGB fragment to the luminal side of the channel, the single channel current and the open probability (3.0% ± 1.0%; $n = 3$) were unchanged (Fig. 1*E*).

Expression of Endogenous Chromogranin and Heterologous Proteins in NGF-treated PC12 Cells—The experiments shown in Fig. 1 demonstrate that the CGB fragment modulates the $InsP_3R$ *in vitro*. We next tested whether this CGB fragment also altered Ca^{2+} signaling *in vivo*. First, the endogenous expression of CGA and CGB in PC12 cells was confirmed. PC12 cells were treated with NGF for 6 days, and the presence of CGA and CGB in cell homogenates was detected using Western blot analysis (Fig. 2*A*). Chinese hamster ovary cells were used as a negative control (Fig. 2*A*).

Second, to verify that NGF-treated PC12 cells would express the CGB fragment as the cells differentiated to a neuron-like morphology, we monitored the level of GFP expression over a 6-day period. The expression of GFP was limited to the ER by using a vector with both ER localization and retention signals along with a Myc epitope (described under "Experimental Procedures"). This same construct was used for the heterologous expression of the CGB fragment discussed below. GFP expression was similar in NGF-treated PC12 cells on days 2, 4, and 6 post-transfection (Fig. 2*B*).

Localization of the CGB Fragment and Scrambled Peptide in NGF-treated PC12 Cells—To express the CGB fragment or the scrambled peptide, PC12 cells were transiently transfected with the vector containing ER localization and retention signals along with a Myc epitope (Fig. 3*A*). After transfection, the cells were treated for 6 days with NGF. To show the localization of the peptide, cells were stained with an anti-Myc antibody. To show the extent of the ER, the cells were co-stained with an

[2] S. N. Jacob, C. U. Choe, M. Yeckel, and B. E. Ehrlich, submitted for publication.

Downloaded from www.jbc.org at Yale University on December 15, 2006
The Journal of Biological Chemistry

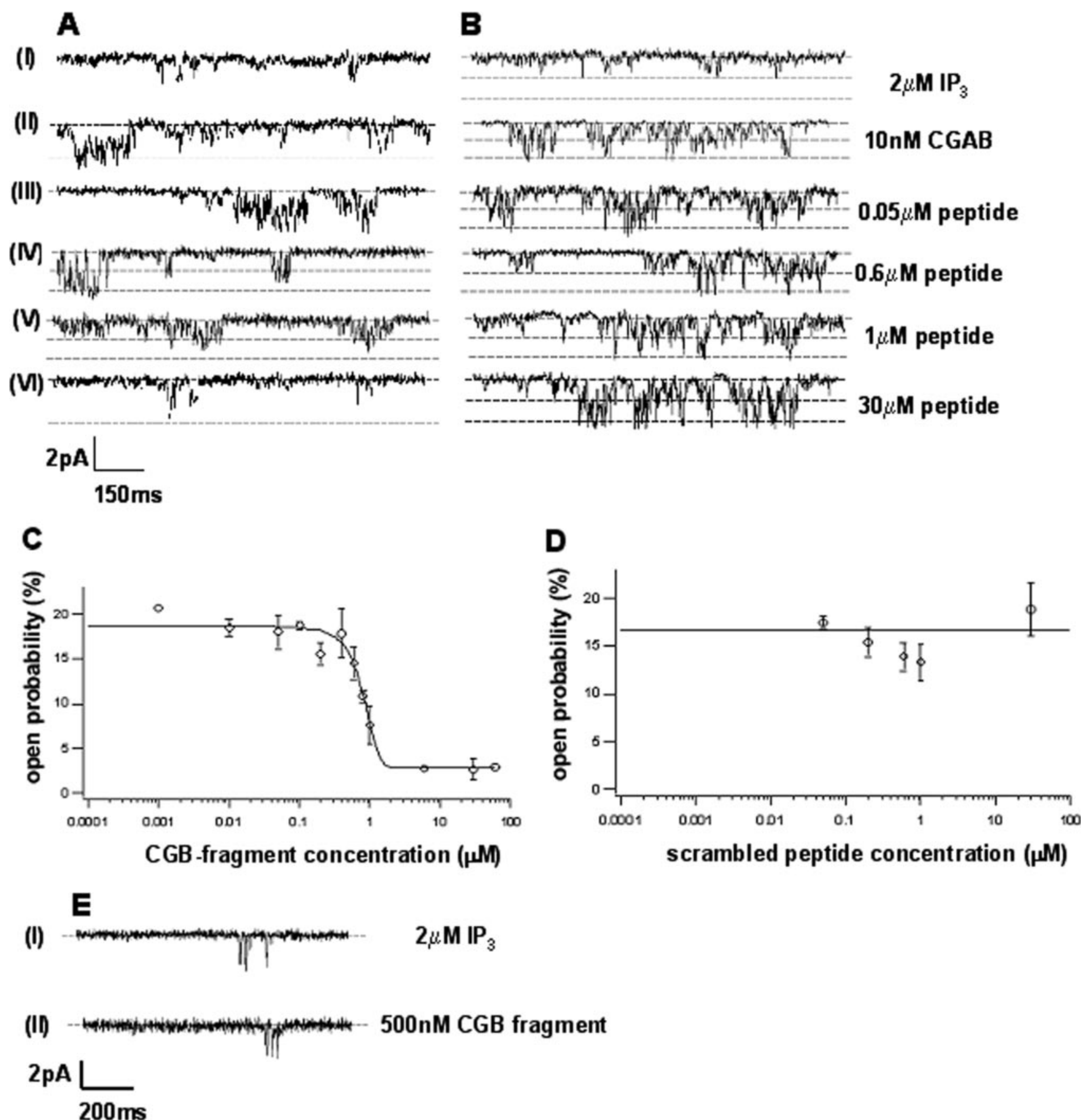

FIG. 1. **Effect of the CGB fragment on $InsP_3$-gated single channel currents.** *A*, *trace I*, $InsP_3R$ single channel activated by 2 μM $InsP_3$ added to the cytosolic compartment. *Openings* are defined as downward deflections from the base line. *Trace II* and subsequent traces (conditions are same as for trace I) with the exception of 0.01 μM CGAB were present in the *trans*-compartment at pH 7.35. *Traces III*, *IV*, *V*, and *VI* are at a CGB fragment concentration of 0.05, 0.6, 1.0, and 30.0 μM, respectively. *B*, same conditions as in A except that a scrambled peptide was used instead of the CGB fragment. *C*, absolute open probabilities of CGAB and $InsP_3$-activated $InsP_3R$ were plotted against the corresponding CGB fragment concentrations. Values could be fitted to a sigmoid function with an IC_{50} of 470 nM (n = 4). *D*, absolute open probabilities of 10 nM CGAB and 2 μM $InsP_3$-activated $InsP_3R$ were plotted against the corresponding scrambled peptide concentrations. Data has been fitted to a linear function (n = 3). *E*, *trace I*, $InsP_3R$ were activated with 2 μM $InsP_3$. *Openings* are defined as downward deflections from the base line. *Trace II*, single channel recording with 500 nM CGB fragment in the luminal compartment (n = 3). All of the values are presented as the mean ± S.E.

anti-calnexin antibody. Fluorescence signals for both compounds were found throughout the soma and the neurites (Fig. 3*B*). The overlap of the signals (Fig. 3*B*, *right panel*) suggests that the two proteins are in the same compartment, which the calnexin stain reveals to be the ER.

Inhibition of the CGAB/InsP₃R Interaction Results in Decreased Ca^{2+} Signaling—To investigate the effect of the CGB fragment *in vivo*, PC12 cells were transiently co-transfected with a DsRed construct and the empty vector, the CGB fragment, or the scrambled peptide (Fig. 3*A*). The CGB fragment and scrambled peptide were identical to the peptides used for bilayer experiments. After NGF treatment for 6 days, only differentiated and transfected PC12 cells, which were well loaded with the Ca^{2+} indicator dye, were used for Ca^{2+} imaging (Fig. 4*A*). Cells were stimulated for 2 min with 500 μM carbachol, and the magnitude of the Ca^{2+} rise over the soma was measured (Fig. 4*B*). The peak Ca^{2+} amplitude (F_{max}/F_0) in cells transfected with the empty vector was 2.4 ± 0.3 (n = 11), and

Downloaded from www.jbc.org at Yale University on December 15, 2006

for cells transfected with the scrambled peptide, the peak Ca^{2+} amplitude was 2.2 ± 0.2 (Fig. 4*B*; $n = 13$). There was no significant difference between these two groups of cells (Fig. 4*C*; $p = 0.84$). In contrast, transfection with the CGB fragment elicited peak Ca^{2+} amplitudes of 1.6 ± 0.1 ($n = 17$), a response that was significantly different from cells expressing the scrambled peptide ($p < 0.03$) or cells transfected with the empty vector ($p < 0.04$) (Fig. 4, *B* and *C*).

To test whether the intracellular calcium stores were intact and viable, PC12 cells transiently transfected with either the empty vector or the CGB fragment were stimulated with 10 mM caffeine or treated with thapsigargin. The percent of cells responding to stimulation by caffeine was similar for cells transfected with the empty vector (55%) and those transfected with the CGB fragment (53%). The amplitude and time course for the Ca^{2+} transient also was similar for cells transfected with the empty vector ($n = 11$) or with CGB fragment ($n = 19$) (Fig. 5, *A* and *B*). Similarly, the amplitude and time course for the Ca^{2+} transient was similar after the addition of thapsigargin. These results show that the stores were fully functional, even after transient expression of a protein fragment of CGB in the lumen of the ER.

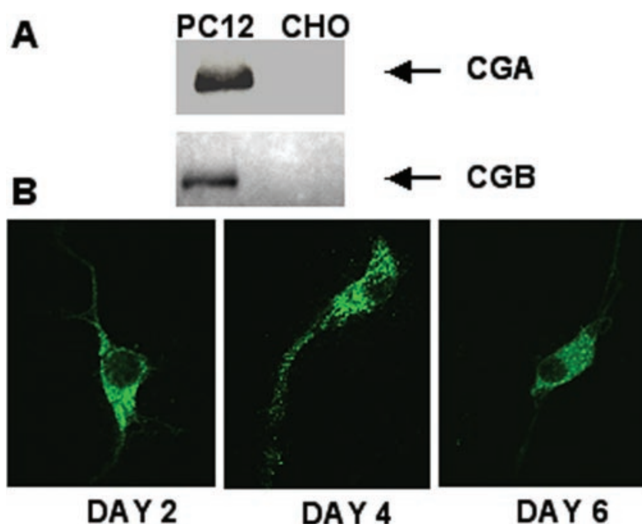

FIG. 2. **Expression of CGA, CGB, and GFP in differentiated PC12 cells.** *A*, Western blot of a homogenate of PC12 cells made from cells treated with NGF for 6 days detected one clear band for CGA and CGB. *B*, PC12 cells were transfected with a GFP-Myc construct with an ER-retention signal and were visualized on days 2, 4, and 6 of NGF treatment.

DISCUSSION

In this paper, we describe experiments where the addition of a CGB fragment was tested on the InsP$_3$R channel activity and on Ca^{2+} signaling in intact cells. We found that the CGB fragment alone does not elicit the effect of the full-length CGAB on the open probability of the InsP$_3$R but that it does inhibit the functional interaction of CGAB on the InsP$_3$R. The inhibition was determined to be specific, because a scrambled peptide with the same amino acid composition as the CGB fragment had no effect (Fig. 1, *B* and *D*). Because the molecular interaction of the CGB fragment with the InsP$_3$R has been shown (27), it is reasonable to conclude that the inhibitory effect is the result of competition between the CGB fragment and CGAB for the same binding site on the InsP$_3$R. Therefore, the CGB fragment can be used as a specific tool to inhibit the functional coupling of CGAB with the InsP$_3$R.

Similarly, the expression of the CGB fragment in neuronally differentiated PC12 cells resulted in a reduction in the peak amplitude of the Ca^{2+} signal after stimulation of PC12 cells with carbachol (Fig. 4, *B* and *C*). Given the single channel results and the presence of intact intracellular calcium stores in transfected PC12 cells, the CGB fragment appears to inhibit the functional coupling of CGAB to the luminal portion of the InsP$_3$R (Fig. 5). This finding suggests that the interaction of CGAB with the InsP$_3$R is necessary for the normal physiological response and is an important component of Ca^{2+} release from intracellular stores by the InsP$_3$R. Moreover, the potential to modulate the binding of CGAB to the InsP$_3$R suggests a powerful means of regulating Ca^{2+} signaling in this estab-

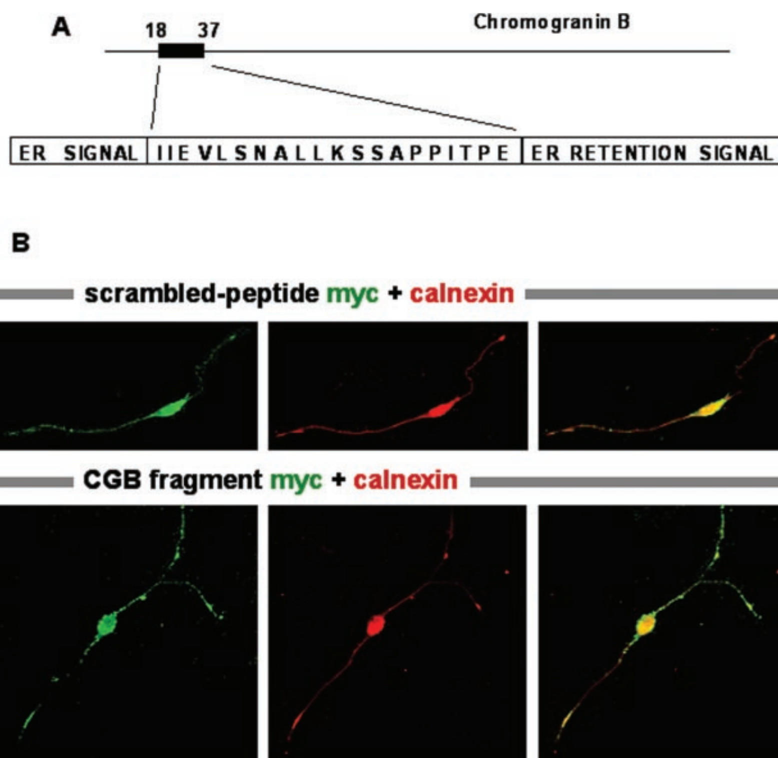

FIG. 3. **ER localization of scrambled peptide and CGB fragment.** *A*, the conserved N-terminally located 20 amino acids of CGB were labeled with an ER signal and an ER retention signal. *B*, double-labeled immunofluorescence of differentiated PC12 cells transfected with scrambled peptide (*upper panels*) or CGB fragment (*lower panels*) stained for the expressed peptide (*green*, *right panels*) or for the well known ER marker, calnexin (*red*, *middle panels*), with merged channels (*right panels*) demonstrating co-localization of the transiently transfected peptides with calnexin.

Downloaded from www.jbc.org at Yale University on December 15, 2006

The Journal of Biological Chemistry

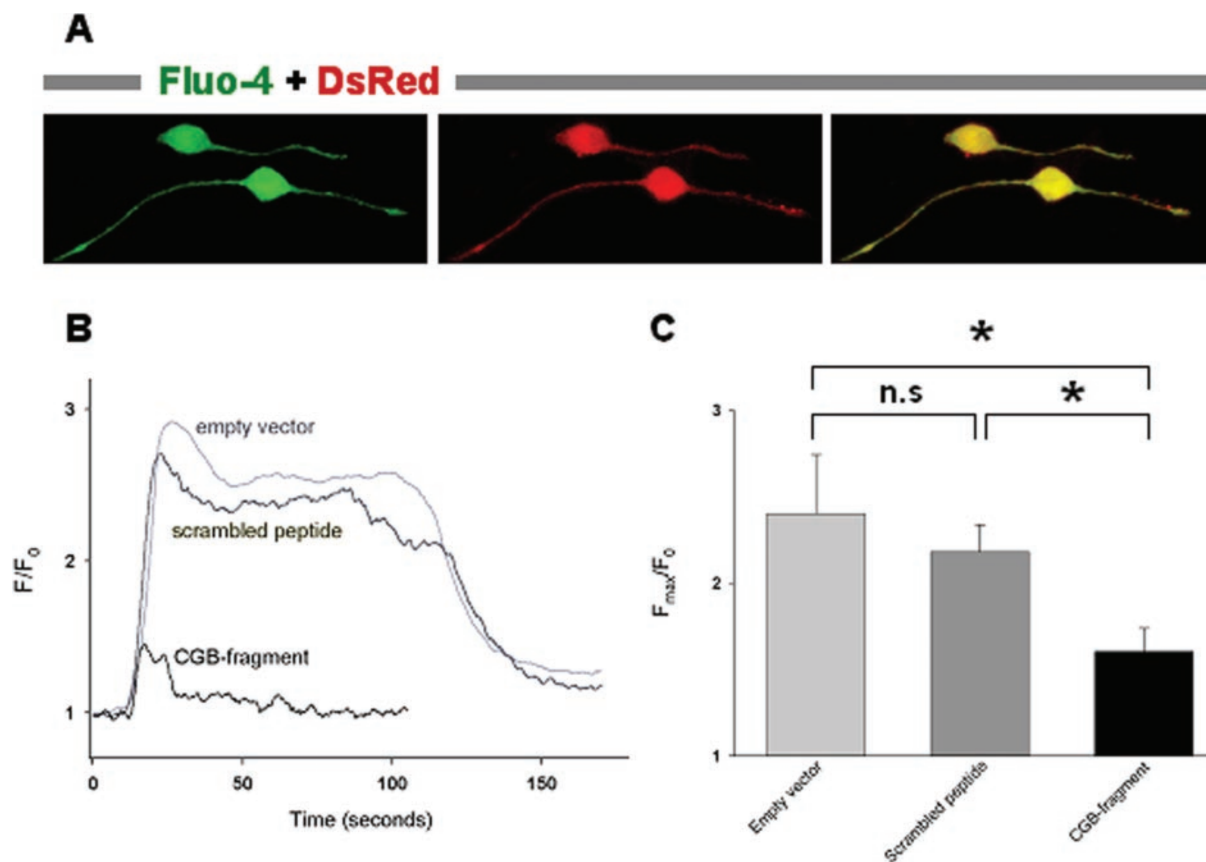

FIG. 4. **Ca^{2+} transients of PC12 cells transfected with CGB fragment, scrambled peptide, or empty vector after stimulation of the $InsP_3R$ pathway.** *A*, differentiated PC12 cells loaded with the Ca^{2+} dye Fluo-4 (*right*) and labeled with DsRed were used for Ca^{2+}-imaging experiments (*middle*). *B*, PC12 cells transfected with the scrambled peptide (*dark gray*) or with the empty vector (*light gray*) showed very similar Ca^{2+}-imaging traces with regard to the amplitude. In comparison, differentiated PC12 cells transfected with the CGB fragment (*black*) elicited much smaller amplitudes. Carbachol was added at 0 s. *C*, differentiated PC12 cells transfected with the empty vector or with the scrambled peptide showed no significant difference concerning their somatic peak Ca^{2+} amplitude (F_{max}/F_0). The CGB fragment expressing PC12 cells had significantly reduced amplitudes compared with either the cells transfected with the empty vector ($p < 0.04$) or with the scrambled peptide ($p < 0.03$). An *asterisk* indicates statistical significance, and all of the values are presented as the mean ± S.E.

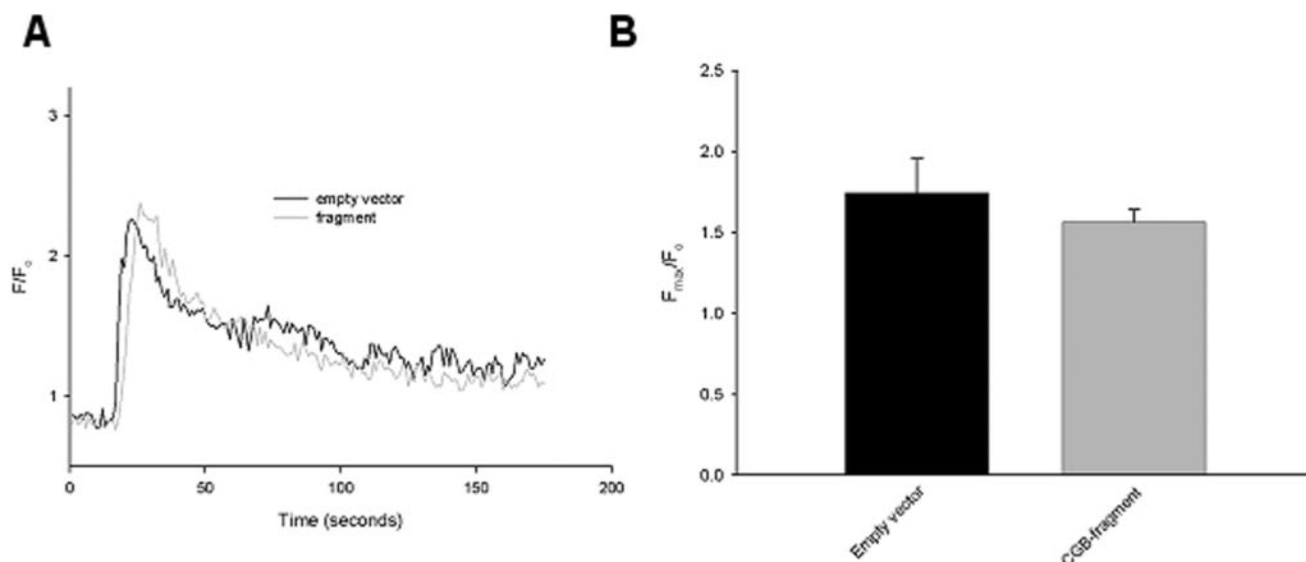

FIG. 5. **Ca^{2+} transients of PC12 cells transfected with CGB fragment or empty vector after stimulation of the ryanodine receptor pathway.** *A*, PC12 cells transfected with the empty vector or with the CGB fragment were stimulated with 10 mM caffeine. The Ca^{2+}-imaging traces were indistinguishable in amplitude and time course, showing that the intracellular Ca^{2+} stores were unaltered by the expression of the CGB fragment. *B*, the amplitudes of the Ca^{2+} transient (F_{max}/F_0) after stimulation by caffeine in PC12 cells transfected with the empty vector ($n = 11$) or with the CGB fragment ($n = 19$). There was no significant difference in the amplitudes.

lished neuronal model (28) and in all cells where these proteins are found. Interestingly, if the expression levels of CGA and CGB are reduced by RNA interference techniques, ER formation is inhibited, showing that the chromogranins are important for ER function (32). Previous studies have shown that the binding of CGAB to the $InsP_3R$ is pH-dependent and that the

Downloaded from www.jbc.org at Yale University on December 15, 2006

functional effects on channel activity correlate with the binding of the two proteins (12, 26). In addition, it is reasonable to assume that very specific subcellular distribution patterns of chromogranin and modulation of its expression will shape Ca^{2+} signal initiation and wave propagation (29).[2]

This binding of the chromogranin to the InsP$_3$R stabilizes the channel properties in a manner similar to that described for the FK506-binding protein 12 association with the ryanodine receptor (33). The increases in single channel mean open time and open probability *in vitro* and the amplification of Ca^{2+} signaling *in vivo* are similar to those seen for the FK 506-binding protein 12/ryanodine receptor complex (33). Assuming that the CGB/InsP$_3$R interaction is modulated and is established as an essential functional channel complex, regulation of InsP$_3$-dependent Ca^{2+} release by CGB provides a new role for the chromogranins.

Although the functional association between chromogranin and the InsP$_3$R is shown here for the type I isoform, it is likely that similar interactions will also occur with the other InsP$_3$R isoforms. The intraluminal loop of the InsP$_3$R essential for chromogranin binding is conserved among the three InsP$_3$R isoforms (34), and all of the InsP$_3$R isoforms bind chromogranins (14). In addition to extending the association of chromogranin to all of the InsP$_3$R isoforms, this relationship can also be generalized to a variety of other cell types. The CGB/InsP$_3$R interaction has most often been associated with endocrine and neuroendocrine cells (35, 36) as well as nonadrenergic neurons of the central nervous system (37). Recently however, both the InsP$_3$R and CGA were found to be selectively expressed in the apical region of ciliated oviductal cells (38) and have been suggested to play a role in controlling the ciliary activity of these cells. In addition to the potential role in ciliary function, it is very likely that the interaction between InsP$_3$R and chromogranin will be the basis for numerous cellular processes, which depend on changes in intracellular Ca^{2+} levels.

In neurons, both of the brain and periphery, a significant alteration in the CGB/InsP$_3$R interaction will lead to dramatic changes not only in the initiation and propagation of Ca^{2+} signals but also in synaptic modulation, the regulation of gene expression, and the development of excitable cells (1). Examples of altered CGB levels have been reported in tissue from patients with neurological diseases. Reduced levels of CGB were found in distinct subregions of the hippocampus from individuals with schizophrenia (39). In contrast, increased levels of CGB were found in the cerebrospinal fluid, Lewy bodies, and axonal swellings of patients with Parkinson's disease (25, 40, 41). A combination of increased and decreased CGB expression levels was found throughout the hippocampus of individuals with Alzheimer's disease where a layer-specific distribution of CGB was present (30, 42). A potential mechanism for the pathophysiology of these neurological diseases is an altered neuronal Ca^{2+} signaling pattern. For example, the elevated CGB expression levels could result in increased InsP$_3$-dependent Ca^{2+} release, which would account for subsequent cellular malfunctioning and apoptosis.

In summary, this study demonstrates the shaping of cellular Ca^{2+} signaling by the interaction of a luminal Ca^{2+} storage protein with an intracellular Ca^{2+} release channel. The complex of these two proteins can now be considered to be major components of the basic functional channel unit, and the modulation of either component will shape InsP$_3$-dependent Ca^{2+} release. The interactions described here also suggest a pathophysiological mechanism for neurological diseases.

Acknowledgments—We thank Dr. R. Fischer-Colbrie for providing us with the cDNA for bovine CGB, Drs. Anton Bennett and Michael Nathanson for thoughtful discussions, and Dr. A. Varshney, G. Anyatonwu, S. N. Jacob, A. F. Bialek, and C. Schlecker for helpful comments on the paper. B. DeGray provided for excellent technical support, and Dr. P. Uhlen receives special thanks for continued support as the laboratory's experimental closer.

REFERENCES

1. Berridge, M. J. (1998) *Neuron* **21,** 13–26
2. Clapham, D. E. (1995) *Cell* **80,** 259–268
3. Ghosh, A., and Greenberg, M. E. (1995) *Science* **268,** 239–247
4. Ehrlich, B. E., Kaftan, E., Bezprozvannaya, S., and Bezprozvanny, I. (1994) *Trends Pharmacol. Sci.* **15,** 145–149
5. Rizzuto, R. (2001) *Curr. Opin. Neurobiol.* **11,** 306–311
6. Simpson, P., Challiss, R., and Nahorski, S. (1995) *Trends Neurosci.* **18,** 299–306
7. Nagase, T., Ito, K., Kato, K., Kaneko, K., Kohda, K., Matsumoto, M., Hoshino, A., Inoue, T., Fujii, S., Kato, H., and Mikoshiba, K. (2003) *Neuroscience* **117,** 821–830
8. Tang, T., Tu, H., Chan, E., Maximov, A., Wang, Z., Wellington, C., Hayden, M., and Bezprozvanny, I. (2003) *Neuron* **39,** 227–239
9. Tang, T., Tu, H., Wang, Z., and Bezprozvanny, I. (2003) *J. Neurosci.* **23,** 403–415
10. Oberdorf, J., Webster, J. M., Zhu, C. C., Luo, S. G., and Wojcikiewicz, R. J. (1999) *Biochem. J.* **339,** 453–461
11. Ando, H., Mizutani, A., Matsu-ura, T., and Mikoshiba, K. (2003) *J. Biol. Chem.* **278,** 10602–10612
12. Thrower, E., Park, H., So, S., Yoo, S., and Ehrlich, B. (2002) *J. Biol. Chem.* **277,** 15801–15806
13. Yoo, S. H. (2000) *Trends Neurosci.* **23,** 424–428
14. Yoo, S., Oh, Y., Kang, M., Huh, Y., So, S., Park, H., and Park, H. (2001) *J. Biol. Chem.* **276,** 45806–45812
15. Simon, J. P., and Aunis, D. (1989) *Biochem. J.* **262,** 1–13
16. Winkler, H., and Fischer-Colbrie, R. (1992) *Neuroscience* **49,** 497–528
17. Tatemoto, K., Efendic, S., Mutt, V., Makk, G., Feistner, G. J., and Barchas, J. D. (1986) *Nature* **324,** 476–478
18. Lewis, J. J., Zdon, M. J., Adrian, T. E., and Modlin, I. M. (1988) *Surgery* **104,** 1031–1036
19. Drees, B. M., Rouse, J., Johnson, J., and Hamilton, J. W. (1991) *Endocrinology* **129,** 3381–3387
20. Aardal, S., Helle, K. B., Elsayed, S., Reed, R. K., and Serck-Hanssen, G. (1993) *J. Neuroendocrinol.* **5,** 405–412
21. Helle, K. B., Serck-Hanssen, G., and Aardal, S. (1993) *Neurochem. Int.* **22,** 353–360
22. Fasciotto, B. H., Trauss, C. A., Greeley, G. H., and Cohn, D. V. (1993) *Endocrinology* **133,** 461–466
23. Mahata, S. K., O'Connor, D. T., Mahata, M., Yoo, S. H., Taupenot, L., Wu, H., Gill, B. M., and Parmer, R. J. (1997) *J. Clin. Investig.* **100,** 1623–1633
24. Strub, J. M., Sorokine, O., Van Dorsselaer, A., Aunis, D., and Metz-Boutigue, M. H. (1997) *J. Biol. Chem.* **272,** 11928–11936
25. Yasuhara, O., Kawamata, T., Aimi, Y., McGeer, E. G., and McGeer, P. L. (1994) *Neurosci. Lett.* **170,** 13–16
26. Thrower, E. C., Choe, C. U., So, S. H., Jeon, S. H., Ehrlich, B. E., and Yoo, S. H. (2003) *J. Biol. Chem.* **278,** 49699–49706
27. Yoo, S. H., and Lewis, M. S. (2000) *J. Biol. Chem.* **275,** 30293–30300
28. Koizumi, S., Bootman, M. D., Bobanovic, L. K., Schell, M. J., Berridge, M. J., and Lipp, P. (1999) *Neuron* **22,** 125–137
29. Johenning, F. W., Zochowski, M., Conway, S. J., Holmes, A. B., Koulen, P., and Ehrlich, B. E. (2002) *J. Neurosci.* **22,** 5344–5353
30. Marksteiner, J., Kaufmann, W. A., Gurka, P., and Humpel, C. (2002) *J. Mol. Neurosci.* **18,** 53–63
31. Thrower, E. C., Mobasheri, H., Dargan, S., Marius, P., Lea, E. J., and Dawson, A. P. (2000) *J. Biol. Chem.* **275,** 36049–36055
32. Huh, Y. H., Jeon, S. H., and Yoo, S. H. (2003) *J. Biol. Chem.* **278,** 40581–40589
33. Brillantes, A.-M. B., Ondrias, K., Scott, A., Kobrinsky, E., Ondriasova, E., Moschella, M. C., Jayaraman, T., Landers, M., Ehrlich, B. E., and Marks, A. R. (1994) *Cell* **77,** 513–523
34. Mikoshiba, K. (1993) *TiPS* **14,** 86–89
35. O'Conner, D. T. (1983) *Regul. Pept.* **6,** 263–280
36. Fischer-Colbrie, R., Lassmann, H., Hagn, C., and Winkler, H. (1985) *Neuroscience* **16,** 547–555
37. Somogyi, P., Hodgson, A., DePotter, R., Fischer-Colbrie, R., Schober, M., Winkler, H., and Chubb, I. (1984) *Brain Res.* **320,** 193–230
38. Steffl, M., Schweiger, M., and Amselgruber, W. (2003) *Ann. Anat.* **185,** 103–108
39. Nowakowski, C., Kaufmann, W., Adlassnig, C., Maier, H., Salimi, K., Jellinger, K., and Marksteiner, J. (2002) *Schizophr. Res.* **58,** 43–53
40. Eder, U., Leitner, B., Kirchmair, R., Pohl, P., Jobst, K., Smith, A., Mally, J., Benzer, A., Riederer, P., Reichmann, H., Saria, A., and Winkler, H. (1998) *J. Neural. Transm.* **105,** 39–51
41. Nishimura, M., Tomimoto, H., Suenaga, T., Nakamura, S., Namba, Y., Ikeda, K., Akiguchi, I., and Kimura, J. (1994) *Brain Res.* **634,** 339–344
42. Marksteiner, J., Lechner, T., Kaufmann, W., Gurka, P., Humpel, C., Nowakowski, C., Maier, H., and Jellinger, K. (2000) *Acta Neuropathol.* **100,** 205–212

Downloaded from www.jbc.org at Yale University on December 15, 2006

ASBMB The Journal of Biological Chemistry jbc