Comparison of and Chromogranin Effect on Inositol 1,4,5-Trisphosphate Sensitivity of Cytoplasmic and Nucleoplasmic Inositol 1,4,5-Trisphosphate Receptor/Ca^{2+} Channels†

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ABSTRACT: The nucleus also contains the inositol 1,4,5-trisphosphate receptor (IP_3R)/Ca^{2+} channels in the nucleoplasm proper independent of the nuclear envelope or the cytoplasm. The nuclear IP_3R/Ca^{2+} channels were shown to be present in small IP_3-dependent nucleoplasmic Ca^{2+} store vesicles, yet no information is available regarding the IP_3 sensitivity of nuclear IP_3R/Ca^{2+} channels. Here, we show that nuclear IP_3R/Ca^{2+} channels are 3−4-fold more sensitive to IP_3 than cytoplasmic ones in both neuroendocrine PC12 cells and nonneuroendocrine NIH3T3 cells. Given the presence of phosphoinositides and phospholipase C and the importance of IP_3-mediated Ca^{2+} signaling in the nucleus, the high IP_3 sensitivity of nuclear IP_3R/Ca^{2+} channels seemed to reflect the physiological needs of the nucleus to finely control the IP_3-dependent Ca^{2+} concentrations. It was further shown that the IP_3R/Ca^{2+} channels of secretory cells are 7−8-fold more sensitive to IP_3 than those of nonsecretory cells. This difference appeared to result from the presence of secretory cell marker protein chromogranins (thus secretory granules) in secretory cells; expression of chromogranins in NIH3T3 cells increased the IP_3 sensitivity of both nuclear and cytoplasmic IP_3R/Ca^{2+} channels by ∼4−6-fold. In contrast, suppression of chromogranin A expression in PC12 cells changed the EC50 of IP_3 sensitivity for cytoplasmic IP_3R/Ca^{2+} channels from 17 to 47 nM, whereas suppression of chromogranin B expression changed the EC50 of cytoplasmic IP_3R/Ca^{2+} channels from 17 to 102 nM and the nuclear ones from 4.3 to 35 nM. Given that secretion is the major function of secretory cells and is under a tight control of intracellular Ca^{2+} concentrations, the high IP_3 sensitivity appears to reflect the physiological roles of secretory cells.

Calcium ions play critical roles in controlling nuclear functions including chromosome replication and transcription control (*1*), and the IP_3-mediated Ca^{2+} release through the inositol 1,4,5-trisphosphate receptor (IP_3R)/Ca^{2+} channels is known to be essential in the assembly of nuclear envelope during cell division (*2*). The IP_3Rs[1] have been shown to be widely present in the nucleoplasm, localizing both in the heterochromatin and euchromatin regions (*3*, *4*). Furthermore, the nucleus of bovine chromaffin cell was recently shown to contain numerous small IP_3-dependent vesicular nucleoplasmic Ca^{2+} stores that consist of the IP_3R/Ca^{2+} channels and Ca^{2+} storage protein chromogranin B (*3*, *5*). These vesicular nucleoplasmic Ca^{2+} stores rapidly released Ca^{2+} in response specifically to inositol 1,4,5-trisphosphate, and other inositol phosphates such as inositol 1,3,4-trisphosphate, inositol 1,4-bisphosphate, or inositol 1,3,4,5-tetrakisphosphate were of no effect (*6*), highlighting the inositol 1,4,5-trisphosphate-dependent nature of the Ca^{2+} stores.

The fact that the IP_3-dependent Ca^{2+} release inside the nucleus is independent of the IP_3-induced Ca^{2+} release from either the nuclear envelope (NE) or the cytoplasm has been previously demonstrated by the results that microinjection of IP_3 into the nucleus of NIH3T3 cells induced Ca^{2+} releases from the nucleoplasm ahead of the NE and the cytoplasm (*7*). On the other hand, when IP_3 was microinjected into the cytoplasm of NIH3T3 cells, the IP_3-induced Ca^{2+} release appeared in the cytoplasm first, which then spread to the NE and the nucleus sequentially. These results and the recent finding of the existence of IP_3-dependent nucleoplasmic Ca^{2+} store vesicles demonstrated the release of Ca^{2+} from the nucleoplasmic Ca^{2+} stores through the IP_3R/Ca^{2+} channels. Although some of the IP_3R/Ca^{2+} channel-containing NE membranes have been reported to penetrate into the nucleoplasm, thus appearing as thin channel- or reticulum-like structures (*8*, *9*), the highly rare presence of NE extensions in the nucleoplasm would have contributed very little, if any, to the IP_3-dependent Ca^{2+} mobilization in the nucleus and is clearly distinguished from the IP_3-sensitive nucleoplasmic Ca^{2+} store vesicles (*5*, *6*).

The cytoplasm also contains IP_3-sensitive Ca^{2+} stores such as the endoplasmic reticulum (ER) (*10*, *11*) and secretory granules (*12*−*14*) that release Ca^{2+} through their IP_3R/Ca^{2+} channels. Since IP_3 is produced from PIP_2 and the amount of PIP_2 available in the cytoplasm and the nucleus is likely

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[1] Abbreviations: IP_3R, inositol 1,4,5-trisphosphate receptor; CGB, chromogranin B; CGA, chromogranin A; NE, nuclear envelope; ER, endoplasmic reticulum.

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to be different (*15*−*17*), the amount of IP_3 produced in the cytoplasm and the nucleus will in all likelihood be different. In light of the importance of IP_3-induced Ca^{2+} mobilization in the control of many critical cytoplasmic and nuclear activities (*1*, *18*), the ability of the cytoplasmic and nucleoplasmic IP_3R/Ca^{2+} channels to open in response to different concentrations of IP_3 will play pivotal roles in determining Ca^{2+} release from each source. In view of the presence of IP_3R/Ca^{2+} channels in the NE (*19*−*21*) and the possibility that the IP_3-dependent nuclear Ca^{2+} mobilization will differ from that of the cytoplasm, past research has centered on the type of IP_3R expressed and on the IP_3 sensitivity of the IP_3R/Ca^{2+} channels localized in the NE and ER using planar lipid bilayer methods (*22*−*24*). However, due to the intrinsic limitation of not being able to look into the IP_3R/Ca^{2+} channels that are located in the nucleoplasm proper (*4*) no information is yet available regarding the sensitivity of the nucleoplasmic IP_3R/Ca^{2+} channels to IP_3.

Given that the difference in the IP_3 sensitivity of these IP_3R/Ca^{2+} channels will dictate the temporal and spatial IP_3-dependent Ca^{2+} concentrations in both the cytoplasm and nucleus, it is of pivotal importance to know the difference in the IP_3 sensitivity of cytoplasmic and nuclear IP_3R/Ca^{2+} channels in order to understand the Ca^{2+} control mechanisms both in and out of the nucleus. We have therefore determined the IP_3 sensitivity of nuclear and cytoplasmic IP_3R/Ca^{2+} channels using both nonneuroendocrine NIH3T3 cells and neuroendocrine PC12 cells. It was thereby shown that the nuclear IP_3R/Ca^{2+} channels were severalfold more sensitive than those of the cytoplasm in both neuroendocrine and nonneuroendocrine cells. Moreover, the IP_3R/Ca^{2+} channels of neuroendocrine cells were significantly more sensitive than those of nonneuroendocrine cells, and this difference appeared to be due to presence of chromogranins (thus secretory granules) in secretory cells. Being the marker proteins of secretory cells (neurons, neuroendocrine cells, exocrine/endocrine cells), chromogranins are absent in nonsecretory cells, probably leading to the lower IP_3 sensitivity of the IP_3R/Ca^{2+} channels of nonsecretory cells. In the present study, the significance of high IP_3 sensitivity of nuclear IP_3R/Ca^{2+} channels and the potential roles of chromogranins in IP_3-dependent Ca^{2+} mobilization mechanisms of secretory cells are also discussed.

EXPERIMENTAL PROCEDURES

Construction of Expression Vectors. The expression vectors for chromogranin A (CGA) and chromogranin B (CGB) were prepared by polymerase chain reaction (PCR) using bovine cDNA as a template, and the PCR products containing full coding sequences were subcloned into the EcoRI/XbaI site of pCI-neo mammalian expression vector (Promega). The expression vectors for CGA- and CGB-ECFP were prepared by subcloning the PCR products into the NheI/SalI site of pd2ECFP-N_1 (Clontech) to produce pd2CGA-ECFP and pd2CGB-ECFP, respectively. Circular plasmid cDNAs for transfection were prepared using a Qiagen maxi-preparation kit.

NIH3T3 Cell Culture and Transient Transfection. All culture reagents were purchased from GibcoBRL, and NIH3T3 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum. Transient transfection was performed with 70−80% confluent cultures, and the cells were transfected with circular plasmid DNAs using LipofecTAMINE-plus transfection reagent (GibcoBRL). Briefly, cells were plated at a density of 5×10^5 cells per well (100 mm in diameter) and were cultured for additional 24 h. Four micrograms of plasmid DNA in 20 μL of LipofecTAMINE plus reagent was mixed with 750 μL of OPTI-MEM I medium and incubated for 15 min at room temperature. In addition, 30 μL of LipofecTAMINE reagent was mixed with 750 μL of OPTI-MEM I and incubated for 15 min. The mixture was then added into a culture plate containing 5 mL of OPTI-MEM I medium.

For real time Ca^{2+} release studies, $\sim 5 \times 10^4$ NIH3T3 cells were plated on a glass coverslip in a well containing 800 μL of OPTI-MEM I, and two DNA transfection reagents, reagents 1 and 2, were prepared. Reagent 1 contained 0.1 μg of plasmid DNA in 6 μL of LipofecTAMINE plus reagent and 100 μL of OPTI-MEM I medium, whereas reagent 2 contained 4 μL of LipofecTAMINE plus reagent and 100 μL of OPTI-MEM I medium. Both reagents were incubated for 15 min at room temperature, followed by mixing of the two reagents and an additional 15 min of incubation. The mixture was then added to the well containing the cells on a coverslip, and the transfection was performed for 3 h at 37 °C. After transfection, the medium was replaced with fresh prewarmed culture medium and was further incubated for 72 h. In our culture condition, about 70−80% of NIH3T3 cells were transfected. The pCI-neo vector or pd2ECFP-N_1 was used as an empty vector. The transfection of CGA- or CGB-ECFP fusion protein was identified on the basis of the cyan fluorescence emission using 425−445 nm excitation and 460−510 nm emission filters, respectively, and microinjection, Ca^{2+} measurements, and the electron microscope experiments were performed using the successfully transfected cells 48 h after transfection.

PC12 Cell Culture and Transient Transfection of CGA- and CGB-siRNAs. PC12 cells were maintained in RPMI 1640 (Gibco BRL) medium supplemented with 10% fetal bovine serum. Transient siRNA transfection was performed with 70−80% confluent cultures. The CGA-siRNA duplex sense and antisense sequences are 5′-CAACAACAACACAGCAGCUdTdT-3′ and 3′-dTdTGUUGUUGUUGUCGUCGA-5′, respectively, and the CGB-siRNA duplex sense and antisense sequences are 5′- AUGCCCUAUCCAAGUCCAGdTdT-3′ and 3′-dTdTUACGGGAUAGGUUCAGGUC-5′, respectively. The 2-nucleotide 3′ overhang of 2′-deoxythymidine is indicated as dTdT. The cells were transfected with the siRNAs using SilencerTM siRNA transfection kit (Ambion). Briefly, approximately $1-2 \times 10^6$ PC12 cells were plated on collagen type IV (BD Biosciences) coated culture dish (100 mm in diameter) in RPMI 1640 medium supplemented with 10% FBS and were cultured for 48 h before transfection. For dose−response experiments of siRNA transfection, 0.25−2 μg of appropriate siRNA and 10 μL of siPORT Amine were used per 5×10^5 cells. But for the EM study, 1 μg of appropriate siRNA and 10 μL of siPORT Amine were used per 5×10^5 cells. Addition of more siRNA did not reduce the number of secretory granules further. The transfection was performed for 6 h at 37 °C. After transfection, the medium was replaced with fresh prewarmed RPMI 1640 medium and was further incubated

for 48 h. The transfection was monitored using a Silencer CyTM3 siRNA labeling kit, and the electron microscope experiments using the transfected PC12 cells were performed 48 h after transfection. With the use of these procedures, the expression of CGA and CGB in the PC12 cells was shown to decrease by ~75−90%, respectively (*25*).

Detection of Nuclear and Cytosolic Ca^{2+} Signals with Confocal Microscopy. Approximately 5×10^4 NIH3T3 cells or $\sim 1 \times 10^5$ PC12 cells that had grown on a glass coverslip were stabilized with OPTI-MEM I medium for 30 min before incubation with the fluorescent Ca^{2+} indicator. Then the cells were treated with a cell permeant fluorescent Ca^{2+} indicator fluo-4/AM (4 μM) in OPTI-MEM I for 40 min at 37 °C, 5% CO_2, after which the cells were washed three times with OPTI-MEM I and then stabilized with the same medium for 30 min at room temperature. The coverslip containing the fluo-4 incubated cells was mounted to a custom-made perfusion chamber on the stage of an inverted microscope (IX71, Olympus). Confocal images of intracellular nuclear and cytosolic Ca^{2+} signals of NIH3T3 and PC12 cells were recorded near the middle of the nucleus using a Perkin-Elmer UltraView LCI confocal imaging system with 60×, 1.4 NA objective lens. In the case of NIH3T3 cells transfected with CGA- or CGB-ECFP, only the cells that emitted ECFP fluorescence were chosen for Ca^{2+} measurement. To detect the confocal fluorescence images of the calcium signals, fluo-4 was excited at 488 nm using an argon laser and a 488/10 nm excitation filter (Chroma Technology Corp. VT), and the emission fluorescence signals were collected through a HQ525/50 nm band-pass filter (Chroma). Images were acquired every 100 ms after microinjection of 10 nM IP_3 (see below), which were analyzed using the region-of-interest (ROI) function of the UltraView LCI Imaging Suite software 5.0 (Perkin-Elmer, Boston, MA). The Ca^{2+} release in the cytoplasm and nucleus of microinjected cells was measured using the UltraView LCI confocal imaging system with a 100× objective (NA = 1.35) from the optical *Z*-section transverse of the middle region of the nucleus of the cell. In these experiments, only the cells uniformly loaded with fluo-4 fluorescence both in the nucleus and the cytosol were used.

Microinjection of IP_3. Microinjection of IP_3 was carried out with an Eppendorf system (Injectman NI2 5181, Femtojet 5247; Eppendorf-Netheler-Hinz, Hamburg, Germany) using pipettes (~100 nm i.d.) pulled from quartz glass (o.d., 1.0 mm; i.d., 0.7 mm, Sutter Instrument) using a P-2000 micropipette puller (Sutter Instrument, Novato, CA). The IP_3 to be microinjected were diluted to their final concentration in a buffer (20 mM HEPES, pH 7.2, 110 mM KCl, 2 mM $MgCl_2$, 5 mM KH_2PO_4, 10 mM NaCl) and filtered with a 0.2 μm filter before filling into the microinjection pipet. Injections were made using the semiautomatic mode of the Eppendorf system at a pipet angle of 45° under the following instrument settings: injection pressure 80 hPa, compensatory pressure 60 hPa, injection time 0.5 s, and velocity of the pipet 2000 μm/s (*26*). Under such conditions using Femtotips II (~500 nm i.d.) as pipettes, the injection volume had previously been estimated to be 1−1.5% of the cell volume in the case of Jurkat T-lymphocytes (*26*). Hence, in light of the similarity in size between Jurkat T-lymphocytes and PC12 cells, and much larger NIH3T3 cells that have an average diameter ~3 times larger than that of PC12 cells, our injection volume was also expected to be ~1% of the PC12 cell volume or less than 0.05% of the NIH3T3 cell volume. Selective microinjection to the nucleus and cytosol was confirmed by microinjection of 4′,6′-diamidino-2-phenylindole (DAPI) and ER-Tracker Blue-White DPX (Molecular Probes, Eugene, OR), respectively. The changes in the fluorescence Ca^{2+} images were acquired every 100 ms.

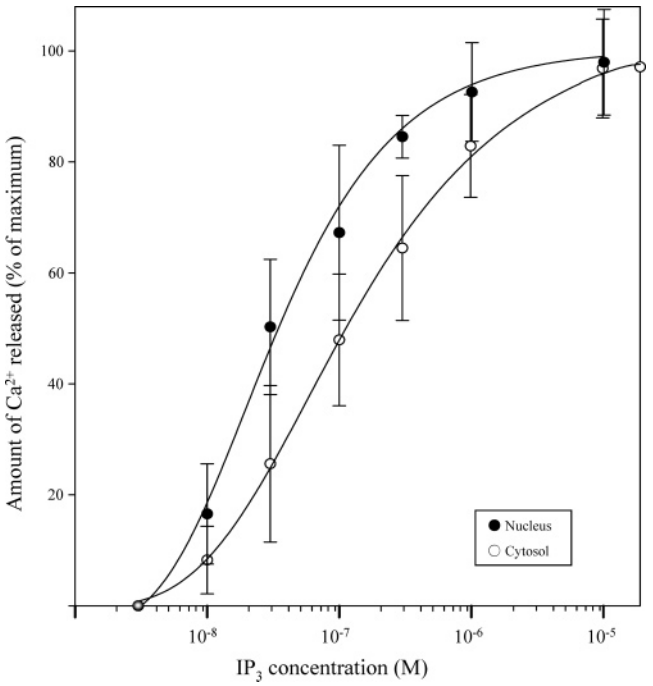

FIGURE 1: IP_3-induced Ca^{2+} releases in the nucleus and cytoplasm as a function of IP_3 concentration in NIH3T3 cells. IP_3-induced Ca^{2+} releases in the nucleus and cytoplasm of NIH3T3 cells were expressed as a function of varying concentrations of microinjected IP_3 into the nucleus and cytoplasm, respectively. The maximum Ca^{2+} release was set at 100%, and Ca^{2+} releases at different IP_3 concentrations were expressed as percentages of the maximum. Each data point shown is mean ± SD of 5−7 independent measurements at the shown IP_3 concentration.

Ca^{2+} Release as a Function of Time. Fluorescence Ca^{2+} signal (F) of the cells was measured over the ROIs drawn in the nucleus, NE, and cytoplasm. The baseline fluorescence (F_o) of each ROI was calculated as the average fluo-4 fluorescence intensity of 100 frames before IP_3 injection. The onset of the Ca^{2+} signal was determined as the time point at which $F - F_o$ began to rise above 5% of the difference between $F_{max} - F_o$ for the first time.

RESULTS

In view of the presence of independent IP_3-sensitive Ca^{2+} stores in the nucleus and cytoplasm and given that different concentrations of IP_3 induce different amounts of Ca^{2+} release, it became of importance to determine the respective IP_3 sensitivity of the IP_3R/Ca^{2+} channels of the nucleus and cytoplasm. Hence, to determine the EC50 values of IP_3 concentration for the nuclear and cytoplasmic IP_3R/Ca^{2+} channels of both secretory and nonsecretory cells, we first measured the IP_3-dependent Ca^{2+} releases as a function of microinjected IP_3 concentration in the nucleus and cytoplasm of nonneuroendocrine NIH3T3 cells (Figure 1). The maximum Ca^{2+} release was set at 100%, and the extent of Ca^{2+} release at a given IP_3 concentration was plotted as a

Table 1: IP_3 Concentration at Which the IP_3-Induced Ca^{2+} Release through the IP_3R/Ca^{2+} Channels Was 50% of the Maximum (EC50 in nM)

	NIH3T3 cell			PC12 cell		
	normal	CGA-ECFP	CGB-ECFP	normal	siCGA	siCGB
nuclear injection	33 ± 11[a]	30 ± 5	6 ± 2	4.3 ± 1.4	4.6 ± 1.4	35 ± 10
cytoplasmic injection	110 ± 35	27 ± 5	20 ± 6	17 ± 5	47 ± 12	102 ± 25

[a] Statistical analyses were performed using one-way analysis of variance, and the EC50 values shown are mean ± SD ($n = 5$). Comparison of the EC50 values between those of nuclear and cytoplasmic IP_3R/Ca^{2+} channels in NIH3T3 and PC12 cells showed $p < 0.001$ by paired t test in all subgroups except the CGA-expressing NIH3T3 cells (CGA-ECFP) in which the difference was not significant.

percentage of the maximum Ca^{2+} release. As shown in Figure 1, injection of 10 nM IP_3 into the nucleus of NIH3T3 cells was sufficient to elicit nuclear Ca^{2+} release, and the amount of Ca^{2+} released continued to increase until ~1−2 μM IP_3 was injected into the nucleus of NIH3T3 cells at which concentrations the amount of Ca^{2+} released no longer increased. On the other hand, the IP_3-induced Ca^{2+} release in the cytoplasm of NIH3T3 cells required higher concentrations of IP_3 than those required in the nucleus and reached its maximum at ~10 μM IP_3 (Figure 1). The EC50 value of IP_3 concentration for the nuclear IP_3R/Ca^{2+} channels of NIH3T3 cells was 33 ± 11 nM (mean ± SD, $n = 5$), whereas that for the cytoplasmic ones was 110 ± 35 nM (Figure 1 and Table 1), demonstrating that the nuclear IP_3R/Ca^{2+} channels are ~3−4-fold more sensitive to IP_3 than the cytoplasmic ones.

To determine the EC50 values of IP_3 concentration for the nuclear and cytoplasmic IP_3R/Ca^{2+} channels of neuroendocrine PC12 cells, we measured the IP_3-dependent Ca^{2+} releases as a function of microinjected IP_3 concentration in both the nucleus and cytoplasm (Figure 2) as described for NIH3T3 cells. As shown in Figure 2, injection of 1 nM IP_3 into the nucleus of PC12 cells began to elicit nuclear Ca^{2+} releases, and the amount of Ca^{2+} released continued to increase until ~0.1 μM IP_3 was injected into the nucleus of PC12 cells at which concentration the amount of Ca^{2+} released no longer increased. On the other hand, the IP_3-induced Ca^{2+} release in the cytoplasm of PC12 cells required higher concentrations of IP_3 than those required in the nucleus and reached its maximum at ~2 μM IP_3 (Figure 2). The EC50 values for the nuclear and cytoplasmic IP_3R/Ca^{2+} channels were 4.3 ± 1.4 nM (mean ± SD, $n = 5$) and 17 ± 5 nM, respectively (Figure 2 and Table 1), indicating that the nuclear IP_3R/Ca^{2+} channels of PC12 cells are ~4-fold more sensitive to IP_3 than the cytoplasmic ones. This result with PC12 cells is consistent with the one shown with NIH3T3 cells and confirms the higher sensitivity of nuclear IP_3R/Ca^{2+} channels. Independent presence and function of the IP_3R/Ca^{2+} channels in the nucleus and cytoplasm are also clearly shown in calcium imaging experiments (Figure 3). Figure 3 shows that in response to 10 nM microinjected IP_3 the initial IP_3-mediated Ca^{2+} release originates in the nucleus when IP_3 is injected into the nucleus (Figure 3A) but in the cytoplasm when IP_3 is injected into the cytoplasm (Figure 3B).

Comparison of the EC50 values of IP_3 concentration not only shows that the nuclear IP_3R/Ca^{2+} channels are ~4-fold more sensitive to IP_3 than the cytoplasmic ones in both neuroendocrine and nonneuroendocrine cells but also indicates that the IP_3R/Ca^{2+} channels in neuroendocrine PC12 cells are ~7−8-fold more sensitive to IP_3 than the corresponding IP_3R/Ca^{2+} channels in nonneuroendocrine NIH3T3 cells. In light of the fact that neuroendocrine cells contain chromogranins, which are the major secretory granule proteins and serve as the granulogenic factors that induce secretory granule formation in the cells they are expressed (*25*, *27*, *28*), and that the presence of chromogranins increases the magnitude of IP_3-dependent Ca^{2+} releases markedly (*29*), the higher IP_3 sensitivity of the IP_3R/Ca^{2+} channels in PC12 cells appeared to be due to the presence of chromogranins in PC12 cells.

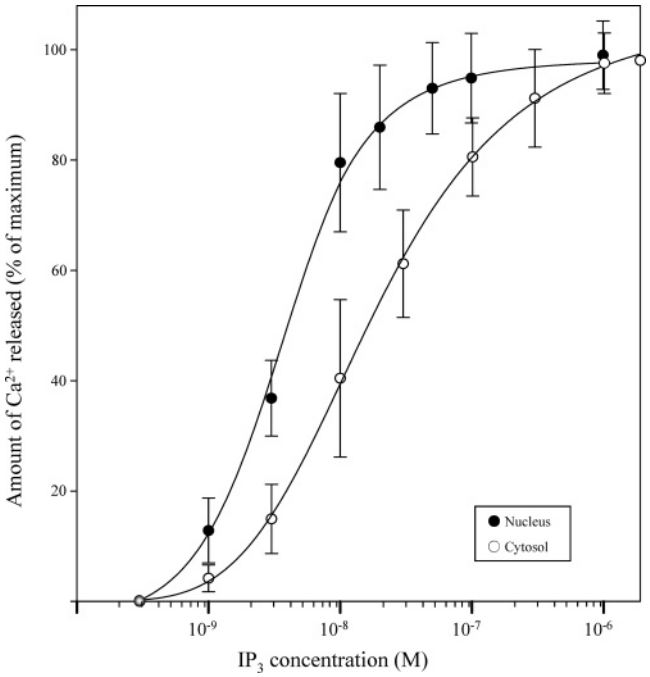

FIGURE 2: IP_3-induced Ca^{2+} releases in the nucleus and cytoplasm as a function of IP_3 concentration in PC12 cells. IP_3-induced Ca^{2+} releases in the nucleus and cytoplasm of PC12 cells were expressed as a function of varying concentrations of microinjected IP_3 into the nucleus and the cytoplasm, respectively. The maximum Ca^{2+} release was set at 100%, and Ca^{2+} releases at different IP_3 concentrations were expressed as percentages of the maximum. Each data point shown is mean ± SD of 5−7 independent measurements at the shown IP_3 concentration.

To determine whether the presence of chromogranins change the IP_3 sensitivity of the IP_3R/Ca^{2+} channels of the cells in which chromogranins are expressed, we expressed CGA and CGB in NIH3T3 cells and tested the IP_3 sensitivity of the IP_3R/Ca^{2+} channels of the chromogranin-expressing NIH3T3 cells (Figures 4 and 7). Determination of the EC50 values of IP_3 concentration for IP_3-induced Ca^{2+} release in the nucleus and cytoplasm of chromogranin-expressing NIH3T3 cells indicated marked differences in the EC50

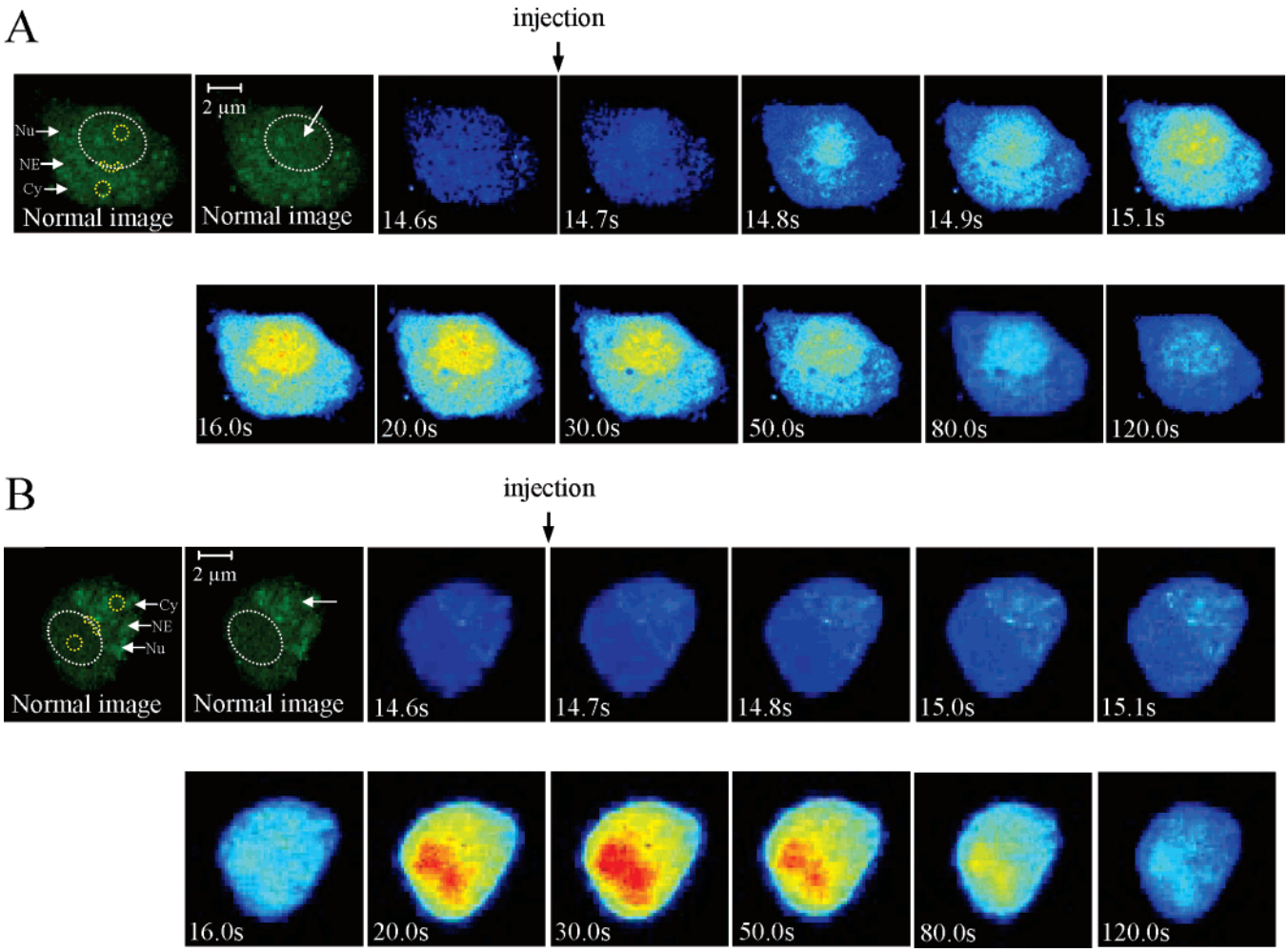

FIGURE 3: Imaging of IP_3-induced Ca^{2+} mobilization in the nucleus and cytoplasm of PC12 cells. An amount of 10 nM IP_3 was microinjected into the nucleus (A) and cytoplasm (B) (the exact location of microinjection is indicated by an arrow within and outside of the demarked nucleus) of PC12 cells at the time indicated by an upward arrow (between 14.6 and 14.7 s). The resulting Ca^{2+} release images are shown as a function of time in pseudocolors. Note that the initial IP_3-dependent Ca^{2+} release is limited to the nucleus (A) and cytoplasm (B), respectively.

values between the chromogranin-expressing and nonexpressing NIH3T3 cells (Figures 4 and 7). The EC50 values of IP_3 concentration in the nucleus and cytoplasm of CGB-expressing NIH3T3 cells were 6 ± 2 nM (mean $\pm$ SD, $n = 5$) and 20 ± 6 nM, respectively (Figure 4, Table 1). These values are not much different from the corresponding EC50 values, 4.3 ± 1.4 nM and 17 ± 5 nM, shown for PC12 cells (Table 1), which appears to reflect the neuroendocrine cell-like nature of the CGB-expressing NIH3T3 cells that contain newly formed secretory granules in the cytoplasm and CGB in the nucleus (*25*).

Calcium imaging results demonstrated clear spatial resolution of the IP_3-induced Ca^{2+} release in the CGB-expressing NIH3T3 cells (Figures 5A and 6A); microinjection of 10 nM IP_3 into the nucleus of CGB-expressing NIH3T3 cells elicited release of Ca^{2+} in the nucleus first, which then spread to the NE and the cytoplasm (Figure 5A). Temporal resolution of the IP_3-induced Ca^{2+} release also confirmed the IP_3-dependent release of Ca^{2+} in the nucleus first (Figure 5, parts B and C). On the other hand, microinjection of 10 nM IP_3 into the cytoplasm of CGB-expressing NIH3T3 cells elicited release of Ca^{2+} in the cytoplasm first, which then spread to the NE and the nucleus (Figure 6A). Temporal resolution of the IP_3-induced Ca^{2+} release also showed the IP_3-dependent release of Ca^{2+} in the cytoplasm first (Figure 6, parts B and C). Comparison of the Ca^{2+} signal slopes of the IP_3-dependent release of Ca^{2+} in the nucleus (Figure 5, parts B and C) and in the cytoplasm (Figure 6, parts B and C) indicates that the diffusion rate of Ca^{2+} signal from the nucleus to the cytoplasm or from the cytoplasm to the nucleus is similar, both Ca^{2+} signals taking $\sim$0.4 s. But the speed of IP_3-dependent Ca^{2+} release in the cytoplasm and nucleus is significantly different; the IP_3-induced Ca^{2+} release in the cytoplasm was fast, reaching the peak level in $\sim$0.2 s, whereas that in the nucleus was relatively slow, continuously releasing Ca^{2+} over an extended period of time after which the released Ca^{2+} began to be sequestered. These results suggest that the cytoplasmic Ca^{2+} concentration can increase very rapidly upon IP_3 introduction, which then is followed by sequestration, while the IP_3-dependent nuclear Ca^{2+} release is a bit slower than that in the cytoplasm but continues for a longer period of time in the nucleus. The apparent difference in the IP_3-dependent Ca^{2+} release pattern of the nucleus and cytoplasm may prove to be essential in carrying out different physiological functions in the respective subcellular location. The same results were also obtained in experiments that had been carried out with the CGA-expressing NIH3T3 cells (not shown). These results further

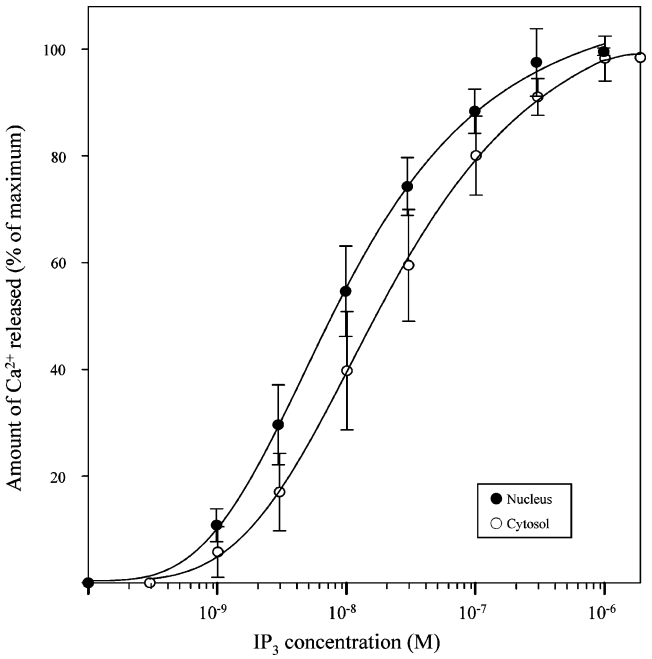

FIGURE 4: IP_3-induced Ca^{2+} releases in the nucleus and cytoplasm as a function of IP_3 concentration in CGB-expressing NIH3T3 cells. IP_3-induced Ca^{2+} releases in the nucleus and cytoplasm of CGB-expressing NIH3T3 cells were expressed as a function of varying concentrations of microinjected IP_3 into the nucleus and the cytoplasm, respectively. The Ca^{2+} releases at different IP_3 concentrations were expressed as percentages of the maximum. Each data point shown is mean ± SD of 5−7 independent measurements at the shown IP_3 concentration.

demonstrated the presence of separate IP_3-dependent Ca^{2+} stores in both the nucleus and cytoplasm that release Ca^{2+} through the IP_3R/Ca^{2+} channels regardless of the expression of chromogranin A or B.

Slightly differing from the CGB-expressing NIH3T3 cells (Figure 4), the EC50 values of IP_3 concentration in the nucleus and cytoplasm of CGA-expressing NIH3T3 cells were 30 ± 5 nM (mean ± SD, $n = 5$) and 27 ± 5 nM, respectively (Figure 7, Table 1). Although the EC50 value in the cytoplasm decreased from 110 ± 35 nM in normal NIH3T3 cells to 27 ± 5 nM in CGA-expressing NIH3T3 cells (Figure 7), a 4-fold increase in the sensitivity of the cytosolic IP_3R/Ca^{2+} channels to IP_3, the EC50 value in the nucleus remained virtually the same, 33 ± 11 nM in the normal cells and 30 ± 5 nM in the CGA-expressing cells (Figure 7), reflecting the lack of CGA expression in the nucleus (*25*, *30*).

Further, in contrast to the expression of chromogranins in normally chromogranin-deficient NIH3T3 cells we have suppressed the expression of intrinsic chromogranins in secretory PC12 cells with siRNA and determined the effect of suppressed chromogranin expression on the IP_3 sensitivity of the IP_3R/Ca^{2+} channels of PC12 cells (Figure 8). In our hands, the siCGA or siCGB treatment resulted in the 75−90% reduction of respective chromogranin expression (*25*), allowing us to determine the chromogranin effect. When the CGA expression was suppressed by siCGA-RNA, the EC50 values of IP_3 concentration for the nuclear and cytoplasmic IP_3R/Ca^{2+} channels of PC12 cells were 4.6 ± 1.4 nM (mean ± SD, $n = 5$) and 47 ± 12 nM, respectively (Figure 8A). The EC50 value of the nuclear IP_3R/Ca^{2+} channels was virtually unchanged, compared to 4.3 ± 1.4 nM of normal PC12 cells. However, the EC50 value of the cytoplasmic IP_3R/Ca^{2+} channels was significantly changed from 17 ± 5 nM (mean ± SD, $n = 5$) to 47 ± 12 nM, ∼3-fold difference. In accordance with the lack of CGA expression in the nucleus, suppression of CGA expression did not affect the EC50 value of the nuclear IP_3R/Ca^{2+} channels of PC12 cells but decreased the IP_3 sensitivity of the cytoplasmic IP_3R/Ca^{2+} channels by ∼3-fold (Table 1).

However, suppression of CGB expression by siCGB-RNA changed the EC50 values of both nuclear and cytoplasmic IP_3R/Ca^{2+} channels of PC12 cells, showing the EC50 value of 35 ± 10 nM (mean ± SD, $n = 5$) for the nuclear IP_3R/Ca^{2+} channels and 102 ± 25 nM for the cytoplasmic ones (Figure 8B). In accordance with the expression of CGB in both the cytoplasm and nucleus, suppression of CGB expression decreased the IP_3 sensitivity of nuclear IP_3R/Ca^{2+} channels by ∼8-fold and that of cytoplasmic ones by ∼6-fold (Table 1), thereby clearly demonstrating the effect of chromogranins on the IP_3 sensitivity of both cytoplasmic and nuclear IP_3R/Ca^{2+} channels of PC12 cells.

DISCUSSION

The IP_3R/Ca^{2+} channels that play major roles in intracellular Ca^{2+} signaling have recently been shown to exist inside the nucleus in addition to their cytoplasmic location in the ER and secretory granules. In accordance with the previous results, the present results demonstrate not only the existence of nuclear IP_3-dependent Ca^{2+} stores in both the neuroendocrine PC12 cells and nonneuroendocrine NIH3T3 cells, independent of the NE or the cytoplasm, but also the difference in IP_3 sensitivity between nuclear and cytoplasmic IP_3R/Ca^{2+} channels. In view of the importance of cytoplasmic and nuclear IP_3R/Ca^{2+} channels to understanding the IP_3-dependent Ca^{2+} control mechanisms of both the cytoplasm and nucleus, and in the absence of information regarding the IP_3 sensitivity of cytoplasmic and nuclear IP_3R/Ca^{2+} channels, the present results provide critical information on the IP_3 sensitivity of IP_3R/Ca^{2+} channels of secretory as well as nonsecretory cells.

As shown in Figures 1 and 2, the nuclear IP_3R/Ca^{2+} channels showed ∼3−4-fold higher IP_3 sensitivity than the cytoplasmic ones in both NIH3T3 cells and PC12 cells. The EC50 values of IP_3 concentration for nuclear and cytoplasmic IP_3R/Ca^{2+} channels of NIH3T3 cells were 33 nM and 110 nM, respectively, whereas the EC50 values of IP_3 concentration of PC12 cells for nuclear and cytoplasmic IP_3R/Ca^{2+} channels were 4.3 and 17 nM, respectively. Interestingly, these values are not much different from the IP_3-binding affinities of either the IP_3-binding amino-terminal 604 residues of three mouse IP_3R isoforms, which showed dissociation constants of 49.5, 14.0, and 163.0 nM (*31*), or the whole three IP_3R isoforms, which showed dissociation constants of 28, 5.8, and 290 nM (*32*), and appear to directly reflect the IP_3-mediated Ca^{2+} releases upon binding of IP_3 to the nuclear and cytoplasmic IP_3R/Ca^{2+} channels.

Given the presence of all three IP_3R isoforms in the cytoplasm and nucleoplasm of NIH3T3 and PC12 cells (*4*, *29*), the higher IP_3 sensitivity of nuclear IP_3R/Ca^{2+} channels is not likely due to predominant localization of any single IP_3R isoform in the nucleus or cytoplasm. Moreover, considering that the resting Ca^{2+} concentrations in the

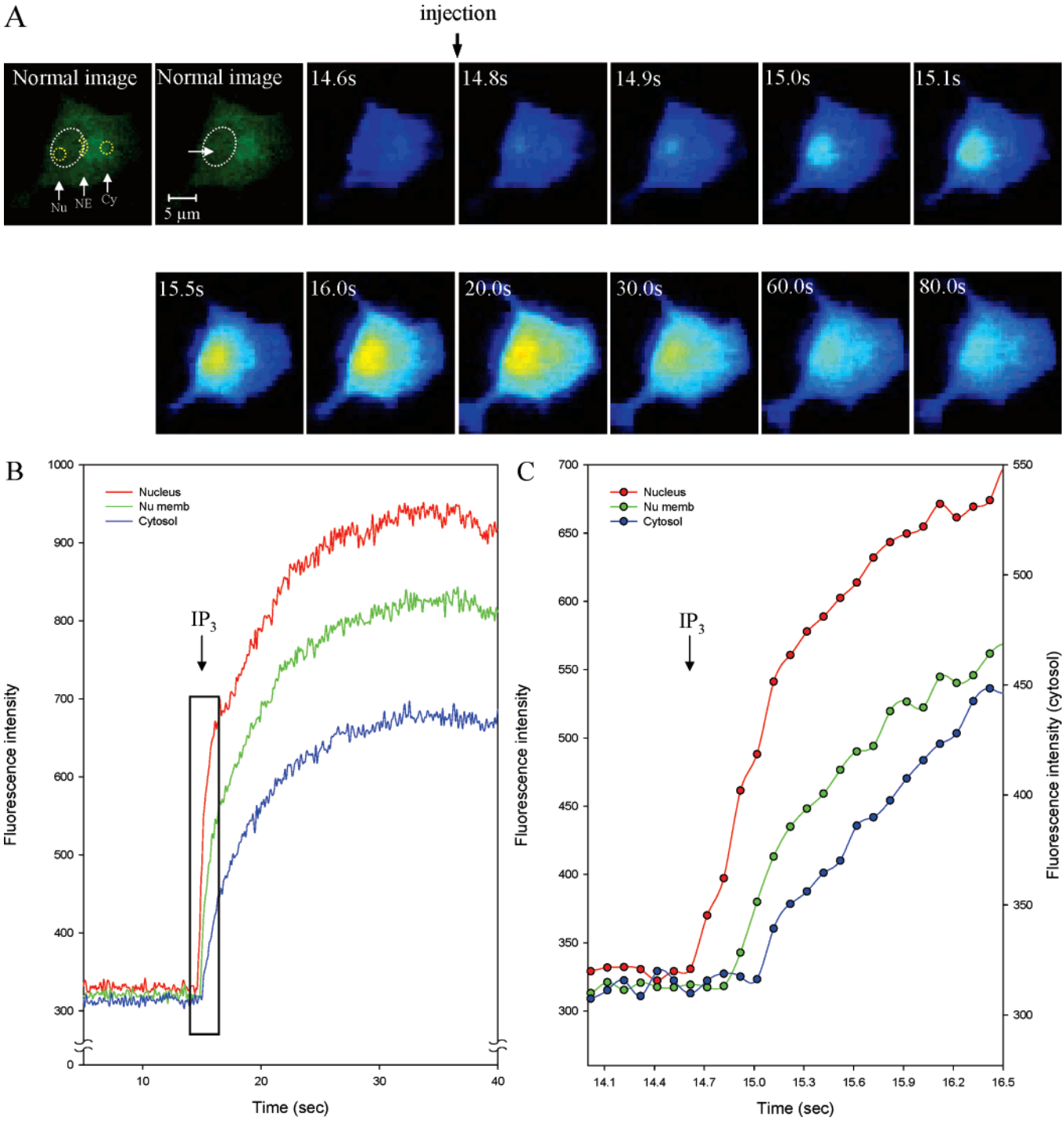

FIGURE 5: Microinjection of IP_3 into the nucleus and Ca^{2+} imaging of CGB-expressing NIH3T3 cells. (A) An amount of 10 nM IP_3 was microinjected into the nucleus (the exact location of microinjection is indicated by an arrow) of CGB-expressing NIH3T3 cells at the time indicated by an upward arrow (between 14.6 and 14.8 s). The resulting Ca^{2+} release images are shown as a function of time in pseudocolors. Note that the initial IP_3-dependent Ca^{2+} release is limited to the nucleus, though it propagated to the cytoplasm later. (B and C) Temporal resolution of the IP_3-induced Ca^{2+} release shows that Ca^{2+} was released first in the nucleus (Nu), followed by the nuclear envelope (NE) and cytoplasm (Cy) of the CGB-expressing NIH3T3 cell. The box shown in (B) is redrawn in (C) in an expanded scale. The subcellular regions from which the Ca^{2+} signals were collected are demarked in the normal image. The results shown are typical of cells microinjected with IP_3 in the nucleus.

nucleoplasm and cytoplasm may not differ greatly, the higher sensitivity of nuclear IP_3R/Ca^{2+} channels is not likely due to activating or inhibitory effect of the Ca^{2+} concentration (*33*−*36*) in the nucleus or cytoplasm (reviewed in *37*). ATP has also been shown to potentiate the IP_3R/Ca^{2+} channel opening (*37*, *38*). In light of the fact that ATP concentration in the cytoplasm is ∼5−10 mM, ∼10% of it being in free form, it appears that the lower IP_3 sensitivity of cytoplasmic IP_3R/Ca^{2+} channels is not due to lower ATP concentration in the cytoplasm. The present results further indicate that regardless of the cell types the nucleus contains the IP_3-sensitive Ca^{2+} stores that are markedly more sensitive to IP_3 than the cytoplasmic ones. Considering that each cell needs to control a vast array of nuclear activities such as NE formation, transcription, and chromosome replication (*1*, *2*, *18*) that are all controlled by nuclear Ca^{2+} concentrations,

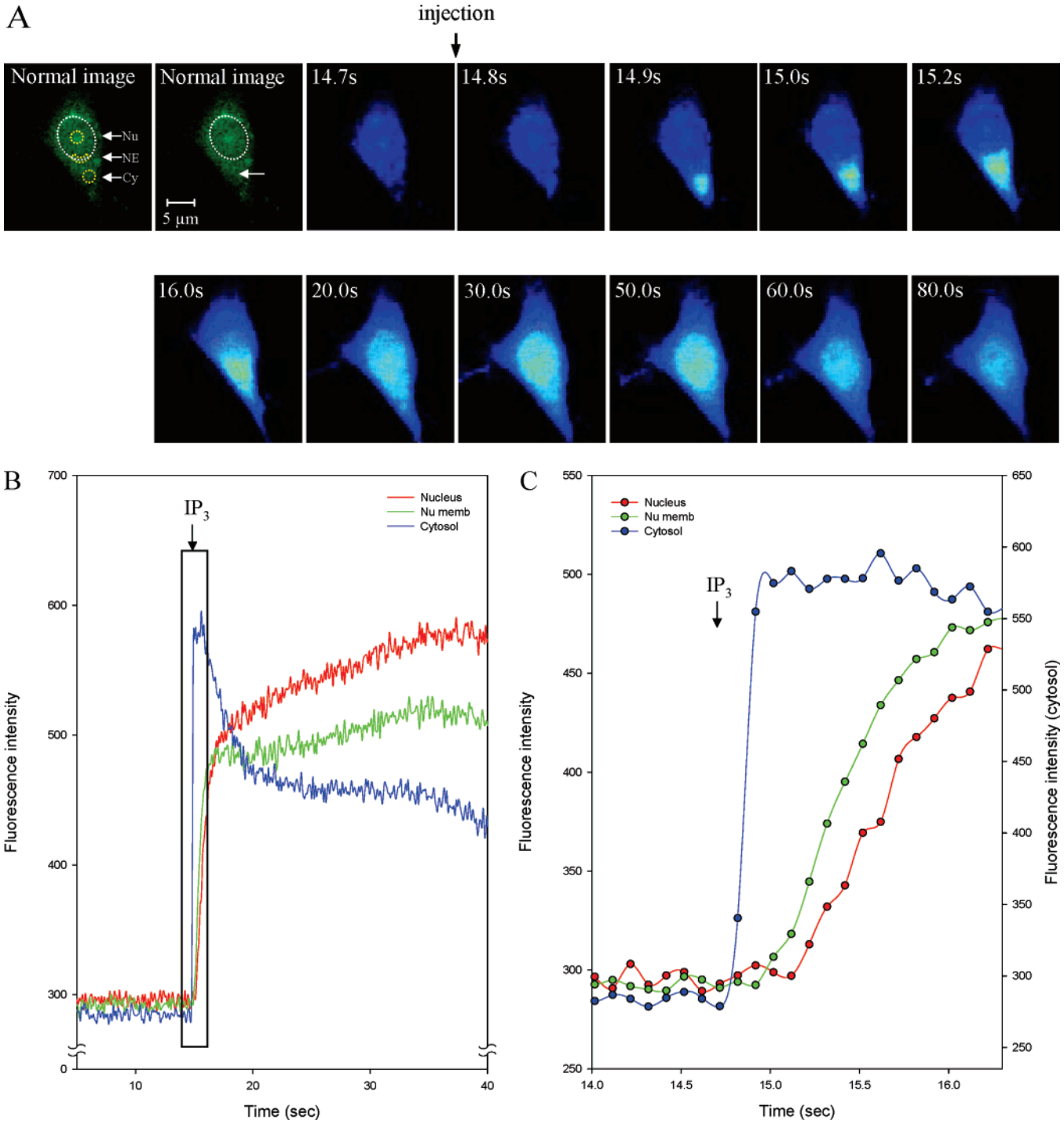

FIGURE 6: Microinjection of IP_3 into the cytoplasm and Ca^{2+} imaging of CGB-expressing NIH3T3 cells. (A) An amount of 10 nM IP_3 was microinjected into the cytoplasm (the exact location of microinjection is indicated by an arrow) of CGB-expressing NIH3T3 cells at the time indicated by an upward arrow (between 14.7 and 14.8 s). The resulting Ca^{2+} release images are shown as a function of time in pseudocolors. Note that the initial IP_3-dependent Ca^{2+} release is limited to the cytoplasm, though it propagated to the nucleus later. (B and C) Temporal resolution of the IP_3-induced Ca^{2+} release shows that Ca^{2+} was released first in the cytoplasm (Cy), followed by the nuclear envelope (NE) and nucleus (Nu) of the CGB-expressing NIH3T3 cell. The box shown in (B) is redrawn in (C) in an expanded scale. The subcellular regions from which the Ca^{2+} signals were collected are demarked in the normal image. The results shown are typical of cells microinjected with IP_3 in the cytoplasm.

the IP_3-dependent nuclear Ca^{2+} control mechanism appears to be well served by the highly sensitive nuclear IP_3R/Ca^{2+} channels.

Although the cytoplasmic IP_3-sensitive Ca^{2+} stores, the ER and secretory granules, and the nucleoplasmic Ca^{2+} store vesicles all share the common property of releasing Ca^{2+} in response to IP_3, the physiological role and size of each organelle in the cell differ significantly. The presumed primary function of secretory granules is storage of secretory cargo and delivery to the extracellular space, whereas that of the ER is modification and folding of proteins, to name a few. Hence, the fact that secretory granules and the ER also function as IP_3-sensitive Ca^{2+} stores (*14*, *39*−*41*) and control the intracellular Ca^{2+} concentration (*42*, *43*) is in line with their roles in the cell. In addition, the ER and secretory granules are significantly larger than the small nucleoplasmic

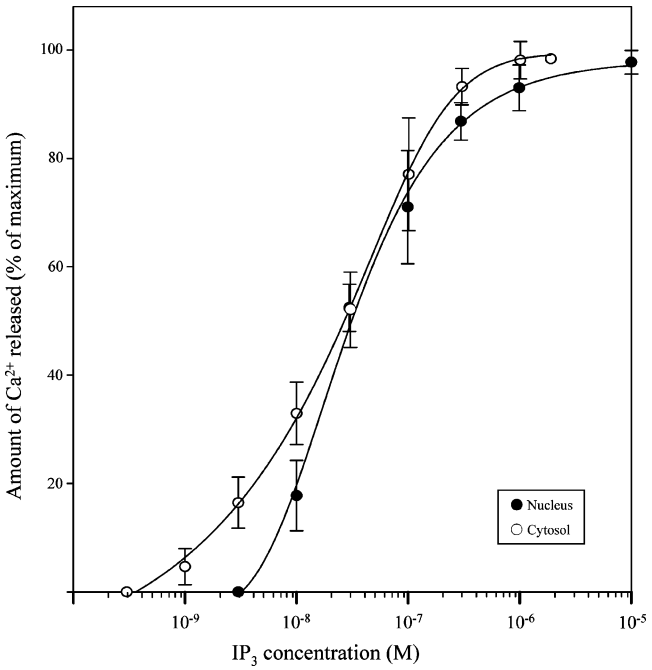

FIGURE 7: IP_3-induced Ca^{2+} releases in the nucleus and cytoplasm as a function of IP_3 concentration in CGA-expressing NIH3T3 cells. IP_3-induced Ca^{2+} releases in the nucleus and cytoplasm of CGA-expressing NIH3T3 cells are expressed as a function of varying concentrations of microinjected IP_3 into the nucleus and the cytoplasm, respectively. Others are the same as Figure 4.

Ca^{2+} stores (50 nm diameter in chromaffin cells) and contain a number of membrane proteins and high concentrations of intragranular/luminal molecules.

The small size of the nucleoplasmic Ca^{2+} store vesicles (50 nm diameter) favors the possibility that these vesicles are specialized for the exclusive purpose of controlling the IP_3-dependent Ca^{2+} concentrations in the nucleus. In view of the fact that the nucleus contains ~11 mM Ca^{2+} (*6*) and the chromosomes contain 20−32 mM Ca^{2+} depending on the condensation state (*44*) and that chromatin and chromosome structures are in dynamic motion (*45*), it would be critical for the small nucleoplasmic Ca^{2+} stores to be highly sensitive to ever-changing IP_3 concentrations in the nucleus. Therefore, the high IP_3 sensitivity of the nuclear IP_3R/Ca^{2+} channels, being 3−4-fold higher than the cytoplasmic ones in both secretory and nonsecretory cells, is in good accord with the potentially important physiological functions of nuclear IP_3R/Ca^{2+} channels.

In previous studies, using HepG2 liver cell line and planar lipid bilayer methods, the EC50 values of IP_3 concentration for the IP_3R/Ca^{2+} channels have been reported to be 64 nM for the NE membranes and 3.7 μM for the ER membranes (*23*). The nuclear membrane EC50 value of 64 nM is relatively close to 33 nM obtained with the nucleoplasmic IP_3R/Ca^{2+} channels of NIH3T3 cells (Figure 1), but 3.7 μM of the ER membranes of HepG2 cells is markedly different from 110 nM of the cytoplasmic IP_3R/Ca^{2+} channels of NIH3T3 cells (Figure 1). The reported EC50 values of the IP_3R/Ca^{2+} channels of the ER membranes, which are obtained by planar lipid bilayer methods, range from 58 nM of type 2 IP_3R channels to 194 nM of type 1 IP_3R channels (*24*) or from 0.5 μM of type 1 IP_3R channels to 3.2 μM of type 3 IP_3R channels (*22*). In spite of the possibility that the EC50 values obtained with the planar lipid bilayer methods

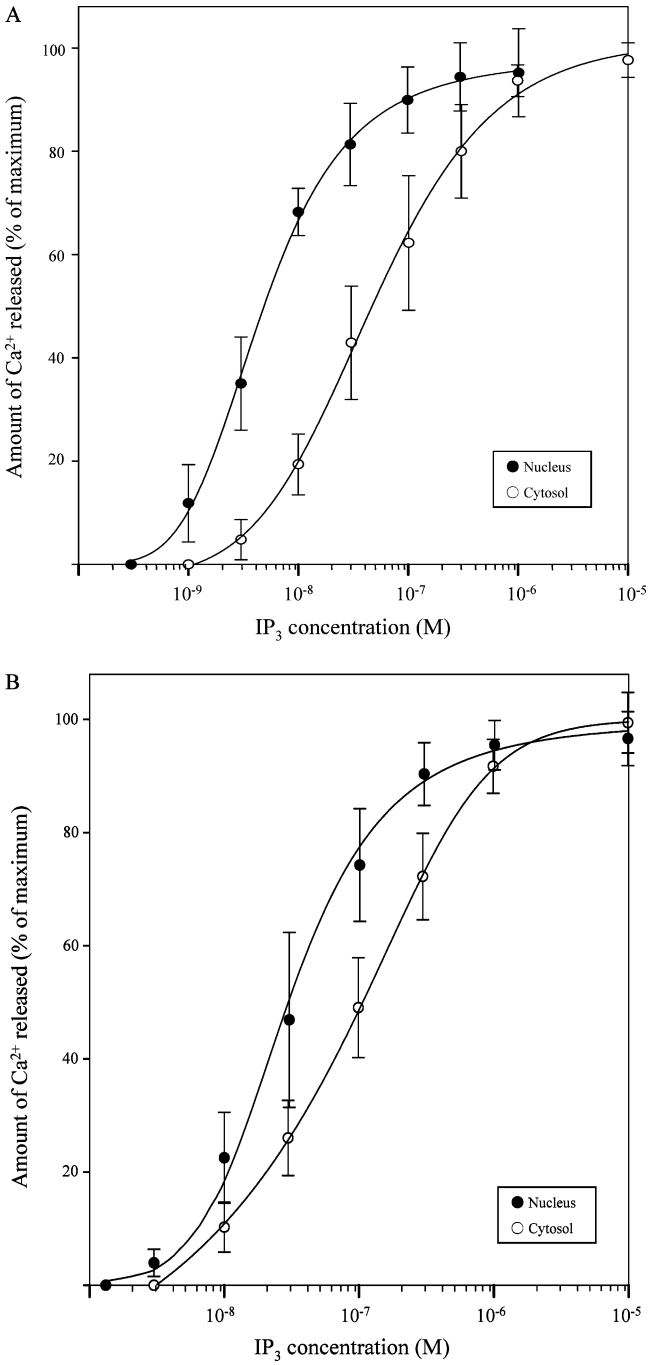

FIGURE 8: IP_3-induced Ca^{2+} releases in the nucleus and cytoplasm as a function of IP_3 concentration in siCGA- and siCGB-treated PC12 cells. IP_3-induced Ca^{2+} releases in the nucleus and cytoplasm of siCGA-treated (A) and siCGB-treated (B) PC12 cells are expressed as a function of varying concentrations of microinjected IP_3 into the nucleus and the cytoplasm, respectively. The Ca^{2+} releases at different IP_3 concentrations are expressed as percentages of the maximum.

may not necessarily indicate the IP_3 sensitivities the IP_3R/Ca^{2+} channels manifest in vivo, the varying results appear to depend on how the membrane vesicles are prepared before reconstitution into the lipid bilayer.

The present results also show that the IP_3R/Ca^{2+} channels of secretory PC12 cells are 7−8-fold more sensitive to IP_3 than those of nonsecretory NIH3T3 cells, in both the nucleus and cytoplasm. The EC50 values obtained with bovine chromaffin cells were even lower than those of PC12 cells (not shown), further underscoring the higher IP_3 sensitivity

of IP_3R/Ca^{2+} channels of secretory cells. The 7−8-fold difference in the IP_3 sensitivity of the IP_3R/Ca^{2+} channels between secretory and nonsecretory cells is much higher than the ~4-fold difference observed between the EC50 values of the nucleus and cytoplasm and, thus, appears to imply relatively more stringent control mechanisms of IP_3-dependent Ca^{2+} concentrations in secretory cells compared to nonsecretory cells. One distinct difference between secretory and nonsecretory cells is the presence of secretory granules and the major secretory granule proteins chromogranins A and B in secretory cells. Given the secretory activity that is under a tight control of intracellular Ca^{2+} concentrations in secretory cells, the 7−8-fold higher sensitivity of the IP_3R/Ca^{2+} channels in secretory cells is likely to reflect the specialized function of these cells. Moreover, unlike secretory cells that need to produce a number of secretory molecules in large quantities rapidly and often, the nonsecretory cells would not need a physiological machinery that operates under an equally sensitive Ca^{2+} control mechanism, probably explaining the lower IP_3 sensitivity of the IP_3R/Ca^{2+} channels in nonsecretory cells.

The major proteins of secretory granules chromogranins A and B (*46*−*48*) play multiple roles in the cell. Not only are chromogranins Ca^{2+} storage proteins, binding 30−90 mol of Ca^{2+}/mol with dissociation constants of 2−4 mM (*49*, *50*), they also couple with the IP_3Rs and activate the IP_3R/Ca^{2+} channels (*51*), increasing the channel open probability 8−16-fold and the mean open time 9−42-fold (*52*, *53*). They are also known to play granulogenic roles, inducing formation of secretory granules (*25*, *27*, *28*) and targeting the IP_3R/Ca^{2+} channels to the granule membrane (*29*). These unique properties of chromogranins seem to be in perfect harmony with the specialized function of secretory cells and their need to control the IP_3-dependent intracellular Ca^{2+} concentrations tightly and appear to serve as the basis for the high sensitivity of the IP_3R/Ca^{2+} channels of secretory cells.

In accordance with the expression of chromogranins in newly formed secretory granules of the chromogranin-transfected NIH3T3 cells (*29*), the IP_3 sensitivity of the cytoplasmic IP_3R/Ca^{2+} channels was substantially increased by expression of either CGA or CGB, increasing 4−6-fold (Figures 4 and 7). Further, in light of the presence of CGB, a member of the IP_3-sensitive nucleoplasmic Ca^{2+} store vesicles (*5*, *6*), in the nucleus and of the coupling of CGB to the IP_3R/Ca^{2+} channel to activate the channel (*53*, *54*), it appears that CGB plays essential roles in the IP_3-induced Ca^{2+} mobilization in the nucleus of secretory cells. Indeed, the nuclear CGB drastically changed the IP_3 sensitivity of nuclear IP_3R/Ca^{2+} channels as evidenced in the CGB-expressing NIH3T3 cells, increasing the IP_3 sensitivity of nuclear IP_3R/Ca^{2+} channels of the CGB-expressing NIH3T3 cells ~6-fold (from the EC50 value of 33 to 6 nM). In contrast, expression of CGA in NIH3T3 cells was without effect on the IP_3 sensitivity of nuclear IP_3R/Ca^{2+} channels (Table 1) because CGA is not expressed in the nucleus (*29*, *30*).

The effect of chromogranins on IP_3 sensitivity of IP_3R/Ca^{2+} channels was further confirmed in PC12 cells that contain intrinsic chromogranins (Figure 8). As shown in Figure 8A, suppression of CGA in PC12 cells decreased the IP_3 sensitivity of cytoplasmic IP_3R/Ca^{2+} channels by ~3-fold (from an EC50 value of 17 to 47 nM) though that of cytoplasmic IP_3R/Ca^{2+} channels remained virtually unchanged (from an EC50 value of 4.3 to 4.6 nM) in accordance with the absence of CGA expression in the nucleus. On the other hand, suppression of CGB expression decreased the IP_3 sensitivity of both cytoplasmic and nuclear IP_3R/Ca^{2+} channels of PC12 cells, decreasing the IP_3 sensitivity of nuclear IP_3R/Ca^{2+} channels ~8-fold (from an EC50 value of 4.3 to 35 nM) and that of cytoplasmic ones ~6-fold (from an EC50 value of 17 to 102 nM). These results clearly demonstrate the profound roles chromogranins play in controlling the IP_3R/Ca^{2+} channel activities in both the cytoplasm and nucleus of cells in which they are expressed.

In view of the regulation of IP_3R/Ca^{2+} channels by Ca^{2+}, IP_3, and ATP (*33*−*36*, *38*, *55*), modulating the channel opening rate (reviewed in *37*), the effect of chromogranins on the IP_3 sensitivity of IP_3R/Ca^{2+} channels appears to be significantly greater than that exerted by Ca^{2+}, IP_3, and ATP. Chromogranin coupling to the IP_3Rs in the presence of Ca^{2+} leads not only to a large conformational change of the coupled chromogranin−IP_3Rs but also to more stable and ordered structures (*56*, *57*). This conformational change of the IP_3R/Ca^{2+} channels to more stable and ordered structures may override any conformational changes caused by Ca^{2+}, IP_3, and ATP available at or near the IP_3R/Ca^{2+} channels and maintain the channels in an "open-ready" condition that can readily open in response to IP_3 binding to release Ca^{2+}.

In spite of the significantly higher IP_3 sensitivity of the IP_3R/Ca^{2+} channels of secretory cells that express chromogranins, it is nevertheless clear that the presence of CGB in the nucleus is not universally required for the nuclear IP_3R/Ca^{2+} channels to be more sensitive to IP_3 from the fact that the nuclear IP_3R/Ca^{2+} channels of nonsecretory NIH3T3 cells are still ~4-fold more sensitive to IP_3 than the counterparts in the cytoplasm. NIH3T3 cells that do not contain intrinsic chromogranins also released Ca^{2+} in response to IP_3 in the nucleus (Figure 1), independent of the NE or the cytoplasm, demonstrating the presence of IP_3-sensitive Ca^{2+} store in the nucleus of nonsecretory cells. It is therefore quite likely that molecules functionally equivalent to CGB of secretory cells might play similar roles in nonsecretory cells.

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REFERENCES

1. Berridge, M. J., Lipp, P., and Bootman, M. D. (2000) The versatility and universality of calcium signalling, *Nat. Rev. Mol. Cell Biol. 1*, 11−21.
2. Sullivan, K. M., Busa, W. B., and Wilson, K. L. (1993) Calcium mobilization is required for nuclear vesicle fusion in vitro: implications for membrane traffic and IP_3 receptor function, *Cell 73*, 1411−1422.
3. Huh, Y. H., Yoo, J. A., Bahk, S. J., and Yoo, S. H. (2005) Distribution profile of inositol 1,4,5-trisphosphate receptor isoforms in adrenal chromaffin cells, *FEBS Lett. 579*, 2597−2603.
4. Huh, Y. H., and Yoo, S. H. (2003) Presence of the inositol 1,4,5-triphosphate receptor isoforms in the nucleoplasm, *FEBS Lett. 555*, 411−418.
5. 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/Ca^{2+} channel, chromogranin B, and phospholipids, *Biochemistry 44*, 9246−9254.

6. Huh, Y. H., Huh, S. K., Chu, S. Y., Kweon, H. S., and Yoo, S. H. (2006) Presence of a putative vesicular inositol 1,4,5-trisphosphate-sensitive nucleoplasmic Ca^{2+} store, *Biochemistry 45*, 1362−1373.
7. Huh, Y. H., Chu, S. Y., Park, S. Y., Huh, S. K., and Yoo, S. H. (2006) Role of nuclear chromogranin B in inositol 1,4,5-trisphosphate-mediated nuclear Ca^{2+} mobilization, *Biochemistry 45*, 1212−1226.
8. Echevarria, W., Leite, M. F., Guerra, M. T., Zipfel, W. R., and Nathanson, M. H. (2003) Regulation of calcium signals in the nucleus by a nucleoplasmic reticulum, *Nat. Cell Biol. 5*, 440−446.
9. Fricker, M., Hollinshead, M., White, N., and Vaux, D. (1997) Interphase nuclei of many mammalian cell types contain deep, dynamic, tubular membrane-bound invaginations of the nuclear envelope, *J. Cell Biol. 136*, 531−544.
10. Meldolesi, J., and Pozzan, T. (1998) The endoplasmic reticulum Ca^{2+} store: a view from the lumen, *Trends Biochem. Sci. 23*, 10−14.
11. Streb, H., Irvine, R. F., Berridge, M. J., and Schulz, I. (1983) Release of Ca^{2+} from a nonmitochondrial intracellular store in pancreatic acinar cells by inositol-1,4,5-trisphosphate, *Nature 306*, 67−69.
12. Gerasimenko, O. V., Gerasimenko, J. V., Belan, P. V., and Petersen, O. H. (1996) Inositol trisphosphate and cyclic ADP-ribose-mediated release of Ca^{2+} from single isolated pancreatic zymogen granules, *Cell 84*, 473−480.
13. Nguyen, T., Chin, W. C., and Verdugo, P. (1998) Role of Ca^{2+}/K^+ ion exchange in intracellular storage and release of Ca^{2+}, *Nature 395*, 908−912.
14. Yoo, S. H., and Albanesi, J. P. (1990) Inositol 1,4,5-trisphosphate-triggered Ca^{2+} release from bovine adrenal medullary secretory vesicles, *J. Biol. Chem. 265*, 13446−13448.
15. Divecha, N., Banfic, H., and Irvine, R. F. (1993) Inositides and the nucleus and inositides in the nucleus, *Cell 74*, 405−407.
16. D'Santos, C. S., Clarke, J. H., and Divecha, N. (1998) Phospholipid signalling in the nucleus, *Biochim. Biophys. Acta 1436*, 201−232.
17. Irvine, R. F. (2003) Nuclear lipid signalling, *Nat. Rev. Mol. Cell Biol. 4*, 349−360.
18. Berridge, M. J. (2005) Unlocking the secrets of cell signaling, *Annu. Rev. Physiol. 67*, 1−21.
19. Gerasimenko, O., and Gerasimenko, J. (2004) New aspects of nuclear calcium signalling, *J. Cell Sci. 117*, 3087−3094.
20. Gerasimenko, J. V., Maruyama, Y., Yano, K., Dolman, N. J., Tepikin, A. V., Petersen, O. H., and Gerasimenko, O. V. (2003) NAADP mobilizes Ca^{2+} from a thapsigargin-sensitive store in the nuclear envelope by activating ryanodine receptors, *J. Cell Biol. 163*, 271−282.
21. Matter, N., Ritz, M. F., Freyermuth, S., Rogue, P., and Malviya, A. N. (1993) Stimulation of nuclear protein kinase C leads to phosphorylation of nuclear inositol 1,4,5-trisphosphate receptor and accelerated calcium release by inositol 1,4,5-trisphosphate from isolated rat liver nuclei, *J. Biol. Chem. 268*, 732−736.
22. Hagar, R. E., and Ehrlich, B. E. (2000) Regulation of the type III InsP(3) receptor by InsP(3) and ATP, *Biophys. J. 79*, 271−278.
23. Leite, M. F., Thrower, E. C., Echevarria, W., Koulen, P., Hirata, K., Bennett, A. M., Ehrlich, B. E., and Nathanson, M. H. (2003) Nuclear and cytosolic calcium are regulated independently, *Proc. Natl. Acad. Sci. U.S.A. 100*, 2975−2980.
24. Ramos-Franco, J., Fill, M., and Mignery, G. A. (1998) Isoform-specific function of single inositol 1,4,5-trisphosphate receptor channels, *Biophys. J. 75*, 834−839.
25. Huh, Y. H., Jeon, S. H., and Yoo, S. H. (2003) Chromogranin B-induced secretory granule biogenesis: comparison with the similar role of chromogranin A, *J. Biol. Chem. 278*, 40581−40589.
26. Guse, A. H., Berg, I., da Silva, C. P., Potter, B. V., and Mayr, G. W. (1997) Ca^{2+} entry induced by cyclic ADP-ribose in intact T-lymphocytes, *J. Biol. Chem. 272*, 8546−8550.
27. Beuret, N., Stettler, H., Renold, A., Rutishauser, J., and 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.
28. Kim, T., Tao-Cheng, J. H., Eiden, L. E., and Loh, Y. P. (2001) Chromogranin A, an "on/off" switch controlling dense-core secretory granule biogenesis, *Cell 106*, 499−509.
29. Huh, Y. H., Jeon, S. H., Yoo, J. A., Park, S. Y., and Yoo, S. H. (2005) Effects of chromogranin expression on inositol 1,4,5-trisphosphate-induced intracellular Ca^{2+} mobilization, *Biochemistry 44*, 6122−6132.
30. Yoo, S. H., You, S. H., Kang, M. K., Huh, Y. H., Lee, C. S., and Shim, C. S. (2002) Localization of the secretory granule marker protein chromogranin B in the nucleus. Potential role in transcription control, *J. Biol. Chem. 277*, 16011−16021.
31. Iwai, M., Michikawa, T., Bosanac, I., Ikura, M., and Mikoshiba, K. (2007) Molecular basis of the isoform-specific ligand-binding affinity of inositol 1,4,5-trisphosphate receptors, *J. Biol. Chem. 282*, 12755−12764.
32. Iwai, M., Tateishi, Y., Hattori, M., Mizutani, A., Nakamura, T., Futatsugi, A., Inoue, T., Furuichi, T., Michikawa, T., and Mikoshiba, K. (2005) Molecular cloning of mouse type 2 and type 3 inositol 1,4,5-trisphosphate receptors and identification of a novel type 2 receptor splice variant, *J. Biol. Chem. 280*, 10305−10317.
33. Cardy, T. J., Traynor, D., and Taylor, C. W. (1997) Differential regulation of types-1 and -3 inositol trisphosphate receptors by cytosolic Ca2+, *Biochem. J. 328 (Part 3)*, 785−793.
34. Kaftan, E. J., Ehrlich, B. E., and Watras, J. (1997) Inositol 1,4,5-trisphosphate (InsP3) and calcium interact to increase the dynamic range of InsP3 receptor-dependent calcium signaling, *J. Gen. Physiol. 110*, 529−538.
35. Vanlingen, S., Sipma, H., De, S. P., Callewaert, G., Missiaen, L., De, S. H., and Parys, J. B. (2000) Ca2+ and calmodulin differentially modulate myo-inositol 1,4, 5-trisphosphate (IP3)-binding to the recombinant ligand-binding domains of the various IP3 receptor isoforms, *Biochem. J. 346 (Part 2)*, 275−280.
36. Tu, H., Wang, Z., and Bezprozvanny, I. (2005) Modulation of mammalian inositol 1,4,5-trisphosphate receptor isoforms by calcium: a role of calcium sensor region, *Biophys. J. 88*, 1056−1069.
37. Foskett, J. K., White, C., Cheung, K. H., and Mak, D. O. (2007) Inositol trisphosphate receptor Ca2+ release channels, *Physiol. Rev. 87*, 593−658.
38. Mak, D. O., McBride, S., and Foskett, J. K. (1999) ATP regulation of type 1 inositol 1,4,5-trisphosphate receptor channel gating by allosteric tuning of Ca^{2+} activation, *J. Biol. Chem. 274*, 22231−22237.
39. Gerasimenko, J. V., Sherwood, M., Tepikin, A. V., Petersen, O. H., and Gerasimenko, O. V. (2006) NAADP, cADPR and IP_3 all release Ca^{2+} from the endoplasmic reticulum and an acidic store in the secretory granule area, *J. Cell Sci. 119*, 226−238.
40. Quesada, I., Chin, W. C., and Verdugo, P. (2003) ATP-independent luminal oscillations and release of Ca^{2+} and H^+ from mast cell secretory granules: implications for signal transduction, *Biophys. J. 85*, 963−970.
41. Xie, L., Zhang, M., Zhou, W., Wu, Z., Ding, J., Chen, L., and Xu, T. (2006) Extracellular ATP stimulates exocytosis via localized Ca release from acidic stores in rat pancreatic beta cells, *Traffic 7*, 429−439.
42. Garcia, A. G., Garcia-De-Diego, A. M., Gandia, L., Borges, R., and Garcia-Sancho, J. (2006) Calcium signaling and exocytosis in adrenal chromaffin cells, *Physiol. Rev. 86*, 1093−1131.
43. Thevenod, F. (2002) Ion channels in secretory granules of the pancreas and their role in exocytosis and release of secretory proteins, *Am. J. Physiol. Cell Physiol. 283*, C651−C672.
44. Strick, R., Strissel, P. L., Gavrilov, K., and Levi-Setti, R. (2001) Cation-chromatin binding as shown by ion microscopy is essential for the structural integrity of chromosomes, *J. Cell Biol. 155*, 899−910.
45. Meaburn, K. J., Misteli, T., and Soutoglou, E. (2007) Spatial genome organization in the formation of chromosomal translocations, *Semin. Cancer Biol. 17*, 80−90.
46. Helle, K. B. (2000) The chromogranins. Historical perspectives, *Adv. Exp. Med. Biol. 482*, 3−20.
47. Taupenot, L., Harper, K. L., and O'Connor, D. T. (2003) The chromogranin-secretogranin family, *N. Engl. J. Med. 348*, 1134−1149.
48. Winkler, H., and Fischer-Colbrie, R. (1992) The chromogranins A and B: the first 25 years and future perspectives, *Neuroscience 49*, 497−528.
49. Yoo, S. H., Oh, Y. S., Kang, M. K., Huh, Y. H., So, S. H., Park, H. S., and Park, H. Y. (2001) Localization of three types of the inositol 1,4,5-trisphosphate receptor/Ca^{2+} channel in the secretory

granules and coupling with the Ca^{2+} storage proteins chromogranins A and B, *J. Biol. Chem. 276*, 45806−45812.
50. Yoo, S. H., and Albanesi, J. P. (1991) High capacity, low affinity Ca^{2+} binding of chromogranin A. Relationship between the pH-induced conformational change and Ca^{2+} binding property, *J. Biol. Chem. 266*, 7740−7745.
51. Yoo, S. H., and Jeon, C. J. (2000) Inositol 1,4,5-trisphosphate receptor/Ca^{2+} channel modulatory role of chromogranin A, a Ca^{2+} storage protein of secretory granules, *J. Biol. Chem. 275*, 15067−15073.
52. Thrower, E. C., Park, H. Y., So, S. H., Yoo, S. H., and Ehrlich, B. E. (2002) Activation of the inositol 1,4,5-trisphosphate receptor by the calcium storage protein chromogranin A, *J. Biol. Chem. 277*, 15801−15806.
53. Thrower, E. C., Choe, C. U., So, S. H., Jeon, S. H., Ehrlich, B. E., and Yoo, S. H. (2003) A functional interaction between chromogranin B and the inositol 1,4,5-trisphosphate receptor/Ca^{2+} channel, *J. Biol. Chem. 278*, 49699−49706.
54. Yoo, S. H., So, S. H., Kweon, H. S., Lee, J. S., Kang, M. K., and 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.
55. Mak, D. O., McBride, S., and Foskett, J. K. (2001) ATP-dependent adenophostin activation of inositol 1,4,5-trisphosphate receptor channel gating: kinetic implications for the durations of calcium puffs in cells, *J. Gen. Physiol. 117*, 299−314.
56. Yoo, S. H., and Lewis, M. S. (2000) Interaction of chromogranin B and the near N-terminal region of chromogranin B with an intraluminal loop peptide of the inositol 1,4,5-trisphosphate receptor, *J. Biol. Chem. 275*, 30293−30300.
57. Yoo, S. H., and Lewis, M. S. (1995) Thermodynamic study of the pH-dependent interaction of chromogranin A with an intraluminal loop peptide of the inositol 1,4,5-trisphosphate receptor, *Biochemistry 34*, 632−638.

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