JOURNAL OF CELLULAR PHYSIOLOGY 205:97–106 (2005)

Effect of Selenium-Supplement on the Calcium Signaling in Human Endothelial Cells

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Intracellular Ca^{2+} signaling controls many cellular functions. Understanding its regulation by selenoproteins is essential for understanding the role of selenoproteins in regulating cell functions. The activity of thioredoxin reductase (TrxR), thioredoxin (Trx) content, and the activity of glutathione peroxidase (GPx) in the human endothelial cells cultured in selenium-supplemented medium (refer as Se^{+} cells) was found 70%, 40%, and 20% higher, respectively than those in the cells cultured in normal medium (refer as Se^{0} cells). The intracellular Ca^{2+} signaling initiated by inositol 1,4,5-trisphosphate (IP_3), histamine, thapsigargin (TG), carbonyl cyanide *p*-(tri-fluoromethoxy) phenyl-hydrazone (FCCP), and cyclosporin A (CsA) was investigated in both Se^{+} and Se^{0} cells. It was interestingly found that the higher activity of selenoproteins reduced the sensitivity of IP_3 receptor to the IP_3-triggered Ca^{2+} release from intracellular stores, but enhanced activation of the receptor-coupled phospholipase C in histamine-stimulated Se^{+} cells by showing much more generation of IP_3 and higher elevation of cytosolic Ca^{2+}. The higher selenoprotein activity also reduced susceptibility of the uniporter to the mitochondrial uncoupler, susceptibility of the permeability transition pore (PTP) to its inhibitor, and the vulnerability of endoplasmic reticulum (ER) Ca^{2+}-ATPase to its inhibitor in selenium-supplementing cells. The results suggest that cell calcium signaling is subjected to thiol-redox regulation by selenoproteins. J. Cell. Physiol. 205: 97–106, 2005. © 2005 Wiley-Liss, Inc.

Selenium as an essential biological trace element plays an important metabolic role via selenoproteins (Ganther, 1999). The most famous disease caused by selenium deficiency is Keshan disease found in China. Blood selenium concentrations measured in areas of China where Keshan disease is endemic are around 0.25 μmol/L (21 μg/L) in comparison with 1.2 μmol/L (95 μg/L) in nonendemic areas (Yang et al., 1984). Selenium in selenoproteins is in the form of selenocystein synthesized cotranslationally from serine and selenide by a series of enzymatic reactions dictated by the UGA codon (Berry et al., 1991). For the human umbilical vein endothelial cell (HUVECs), there are at least 30 selenoproteins in the cells, but thioredoxin reductase (TrxR) and glutathione peroxidase (GPx) are the most important selenoproteins in protecting cells from oxidative damage (Miller et al., 2002). TrxR is the major selenoprotein expressed in HUVECs (Anema et al., 1999). Its catalytic activity is changeable when the cells are cultured in a medium containing different concentrations of selenium (Lewin et al., 2002). TrxR along with its substrate thioredoxin (Trx) constitutes one of the most important systems in maintaining redox homeostasis in cells. TrxR reduces Trx that in turn is capable of controlling various cellular redox-related processes such as reducing disulfide on proteins, donating hydrogen for ribonucleotide reductase in DNA synthesis, activating the glucocorticoid receptor (Makino et al., 1999) and chaperones in protein folding. Furthermore, Trx can also reduce H_2O_2 and scavenge free radicals (Spector et al., 1988). GPx is an antioxidant enzyme that scavenges H_2O_2 and organic hydroperoxides, and its expression level depends on selenium availability (Stewart et al., 1999; Helmy et al., 2000; Tolando et al., 2000). The activity of GPx is also important for maintaining cellular thiol homeostasis. An increase in the amount of GPx in selenium-supplemented cells has been shown to inhibit both the activation of mitogen-activated protein kinases such as p38, JNK1, or JNK2 and ERK1 or ERK2 as well as the NF-κB mediated signaling pathway (Adler et al., 1996; Makropoulos et al., 1996; Stapleton et al., 1997; Schieke et al., 1999). Although, many studies on selenoproteins have been devoted to the regulation of various cell functions and intracellular signal transduction events, no study on the role of selenoproteins in regulating Ca^{2+} signaling has been reported.

Changes in the intracellular concentration of calcium control many cellular functions ranging from short-term responses such as muscle contraction and hormone secretion to long-term regulation of cell growth, proliferation, and apoptosis (Berridge et al., 1998). Many cells use inositol 1,4,5-trisphosphate (IP_3), the product of a phosphatidylinositol-specific phospholipase C, as the second messenger to generate intracellular Ca^{2+} signal by binding to the IP_3 receptor (IP_3R), the Ca^{2+} channel on endoplasmic reticulum (ER), which is the major intracellular Ca^{2+} store in many cell types. The signal comprises an initial Ca^{2+} release from ER and a followed Ca^{2+} entry triggered by emptying of intracellular Ca^{2+} store (Berridge et al., 2000; Putney et al., 2001). After Ca^{2+} release, the refilling of Ca^{2+} into intracellular stores is mediated by Ca^{2+}-ATPase on ER (Lytton et al., 1991). PLC, IP_3R, and the Ca^{2+}-ATPase on ER are, therefore, potential targets for modifying the

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Contract grant sponsor: National Natural Science Foundation of China; Contract grant number: 30330250.

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Received 11 December 2004; Accepted 18 January 2005

DOI: 10.1002/jcp.20378

intracellular Ca^{2+} signaling. In addition, the Ca^{2+} signaling is further complicated by other organelles in cells. There is increasing evidence to show that mitochondrion is an active participant in intracellular Ca^{2+} buffering and signaling. Its unique role is able to rapidly accumulate and then release large quantities of Ca^{2+} by detecting cytoplasmic Ca^{2+} signal resulted from the discharge of the ER Ca^{2+} store. Conversely, both the buffering of the cytoplasmic Ca^{2+} and ATP production by mitochondria is predicted to influence the ER Ca^{2+} handling (Landolfi et al., 1998; Murchison and Griffith, 2000). Triggering of the IP_3 receptor on ER membrane raises the Ca^{2+} concentration both in the cytoplasm and in mitochondria, which subsequently activates the metabolic activity according to the increased cell needs, such as more production of NADH (Rizzuto et al., 1994). After detection of depletion of ER calcium store, mitochondria can feed back calcium to ER calcium store. A protein called permeability transition pore (PTP) mediates the efflux of Ca^{2+} from mitochondria. Opening of the PTP forms a major pathway for rapid release of Ca^{2+} from mitochondria (Kidd et al., 2002). Mitochondria act as calcium buffer in cell Ca^{2+} signaling. Besides mitochondria, it has been also known that Ca^{2+} store within Golgi apparatus may also be released in response to IP_3-producing agonists (Pinton et al., 1998). Part of the accumulated Ca^{2+} in the Golgi apparatus can be released by IP_3 (Van Baelen et al., 2001). A PMR1 P-type-like ATPase ion pump was found to transport Ca^{2+} and Mn^{2+} with high affinity into the Golgi apparatus in a thapsigargin (TG)-insensitive manner. Therefore, any oxidized or reduced alteration of those proteins, which functions either as Ca^{2+} channel or ion pump, would be expected to modify the intracellular Ca^{2+} signaling.

Recently, increasing evidence has shown that the activity of Ca^{2+}-ATPases, Na^{2+}/Ca^{2+} exchanger and calmodulin are modulated by the intracellular redox state (Sauer et al., 2001). It may be expected that the redox status of the proteins involved in abovementioned Ca^{2+} signaling would be modulated by selenoproteins, especially TrxR/Trx and GPxs. In the present study, the effect of selenoprotein(s) on Ca^{2+} signaling has been investigated by using selenium-supplemented culture medium to raise the activities of TrxR and GPx in the HUVECs, and using IP_3, histamine, TG, FCCP, and cyclosporin A (CsA) to probe the related proteins involved in Ca^{2+} release from various stores. This study demonstrates a significant role of selenoprotein(s) in regulating Ca^{2+} signaling.

MATERIALS AND METHODS

Reagents

Mag-fura-2/AM and fura-2/AM were obtained from Molecular Probes (Eugene, OR), 1-(6-{[17β-3-methoxyestra-1,3,5(10)-trien-17-yl]amino}hexyl)-1H-pyrrole-2,5-dione (U73122), D-myo-IP_3 hexasodium salt, digitonin, carbonyl cyanide *p*-trifluoromethoxyphenyl hydrazone (FCCP), TG, histamine dihydrochloride, MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide), sodium selenite, insulin (bovine), 5,5′-dithiiobis-2-nitrobenzoic acid (DTNB), phenylmethylsulfonyl fluoride (PMSF), glutathione reductase, reduced glutathione, mercaptosuccinate (MS), β-nicotinamide adenine dinucleotide phosphate (NADPH), and CsA were obtained from Sigma Chemical Co. (St. Louis, MO). Dulbecco's modified eagle medium (DMEM) was obtained from Gibco Life Technologies (Gaithersburg, MD). Guanidine hydrochloride was purchased from Bebco Industries, Inc. (La Marque, TX). D-myo-IP_3 [^{3}H] assay system was purchased from Amersham Bioscience (Piscataway, NJ). The bovine wild type TrxR was from Department of Biochemistry and Biophysics (Karolinska Institute, Sweden). All other reagents were of analytic grade. CsA was dissolved in ethanol. Digitonin, FCCP, and TG were dissolved in fresh dimethyl sulfoxide (DMSO) just before addition into the cell-containing buffer. The final concentration of DMSO in the buffer never exceeded 0.1%.

Cell culture

ECV304 cells (the HUVEC line) were grown in DMEM containing 10% calf serum, 1 g/L D-glucose, 100 μg/ml streptomycin, and 100 U/ml penicillin. The cells cultured in such medium and in the medium supplemented with 1 μM sodium selenite for more than 1 week are referred as Se^0 and Se^+ cells, respectively. Before measurement of cell calcium, the cells were plated in glass-bottom dishes and incubated in the corresponding medium at 37°C overnight. No toxic effect was observed in the cells cultured in the selenium-supplemented medium (see Fig. 3).

Measurement of TrxR activity and protein concentration in cell extract

The cells were lysed with lysis buffer (50 mM potassium phosphate, 1% Triton X-100, 2 mM EDTA and 1 mM PMSF, pH 7.5). After sonication in ice bath, the cell lysate was centrifuged at 15,000*g* for 30 min. The supernatant fraction was heated at 55°C for 5 min, and filtered in a centrifugal filter of 30-kDa cut-off. The retentate was washed three times with PE buffer (50 mM potassium phosphate and 2 mM EDTA, pH 7.5) and used as crude cell extract. TrxR activity in the cell extract was measured by the modified DTNB reduction assay (Hill et al., 1997). The assay mixture contained 5 mM DTNB, 0.2 mM NADPH, 0.1M potassium phosphate, 10 mM EDTA, and 0.2 mg/ml BSA (pH 7.5). Protein levels of the cell extracts were determined using Bio-Rad Protein Assay system. To inhibit TrxR, the cell extracts (0.3 mg protein/ml) was incubated with 20 μM gold thioglucose at room temperature for 20 min before assay. In all assays, 50 μl of the cell extracts was added into 750 μl of the assay mixture and the changes of absorbance at 412 nm were recorded. The TrxR activity in the cell extract was determined by subtracting the DTNB reduction rate in the presence of gold thioglucose from that in the absence of the inhibitor.

Measurement of Trx content in cells

The cell extracts were obtained same as described in TrxR activity assay, except for the filtration of cell lysate supernatant by a centrifugal filter with 10-kDa cutoff. Trx content was measured by Trx-dependent insulin reduction, in which the Trx in cell extract was firstly reduced by bovine wild type TrxR and then reduces the insulin. The numbers of the exposed sulfhydryl groups in the reduced insulin were quantified by DTNB reduction (Wright and Viola, 1998). In the assay, 15 μl cell extract and 10 μl bovine TrxR (50 A_{412}/ml min) were added in 95 μl reaction mixture containing 100 μM insulin, 0.2 mM NADPH, 25 mM KH_2PO_4, and 5 mM EDTA in 0.1M HEPES buffer (pH 7.6). After incubation at 37°C for 10 min, the reaction was terminated with 500 μl of 8M guanidine–HCl in 50 mM HEPES (pH 7.6) containing 1 mM DTNB. For each sample absorbance of DTNB at 412 nm was measured as Trx content in cells using a calibration curve obtained by the same procedure except for that the purified human wild-type Trx of various concentration replaced the cell extract. The sample prepared by addition of the cell extracts and TrxR after incubation of the reaction mixture with the DTNB–guanidine–HCl solution was used as blank for each measurement. According to the calibration curve, 0.1 A_{412} corresponds to 60 ng Trx. The cellular content of Trx is expressed as the net nanogram Trx per microgram protein.

Measurement of GPx activity in cell extract

The cells were lysed according to the same procedures described for the assay of TrxR activity. The GPx activity was assayed by Paglia and Valentine (1979) method with minor modification. The method is based on two coupled reactions: GPx-catalyzed oxidation of glutathione by H_2O_2 and glutathione reductase-catalyzed oxidation of NADPH by oxidized glutathione. Hundred microlitres of the cytosol supernatant

was added to 2.8 ml of the reaction mixture, that comprises 2.525 ml of a 0.1M Tris-HCl (pH 7.2) buffer, 75 μl of 30M glutathione, 100 μl of 6 mM NADPH, and 100 μl of glutathione reductase (0.24 U), and incubated for 5 min at 25°C. The reaction was initiated by adding 100 μl of 30 mM H_2O_2, and the absorbance at 340 nm was recorded for 5 min. To correct for possible interference from other NADPH-oxidizing enzymes, a blank consisting of the complete reaction mixture plus 4 mM MS, an inhibitor of GPx, was run for every sample (Chaudiere et al., 1984). A molar extinction coefficient of 6.22×10^3 M^{-1} cm^{-1} was used to determine NADPH that is oxidized. The GPx activity in cell extract was expressed as the oxidized NADPH nM min^{-1}/mg protein.

Microscopic measurement of cytosolic Ca^{2+} concentration

The cells plated in the glass-bottomed dishes (2×10^5 cells) were incubated with 1 μM fura-2/AM at 37°C for 40 min, and then washed four times either with Ca^{2+} buffer (10 mM HEPES, 145 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.4) or with Ca^{2+}-free buffer, in which 2.5 mM $CaCl_2$ was replaced by 0.5 mM EGTA, to remove free fura-2 and extracellular free calcium. Then 1 ml of a desired buffer was added into the dish and maintained at 37°C for an additional 10 min to allow for de-esterification of the Ca^{2+} indicator. Fura-2 was excited with light from a mercury lamp alternately filtered to 340 or 380 nm. The fluorescence images of the attached cells on the bottom glass were captured every 20 s at emission of 510 nm for 100 msec on an Olympus IX-71 inverted microscope equipped with AquaCosmos Microscopic Image Acquisition and Analysis System provided by Hamamatsu Photonics K.K. (Japan). The digitized fluorescence ratio (F_{340}/F_{380}) images of the cells and the kinetic change of the ratio in each cell were processed on line on a PC computer.

In the imaging system used in this investigation, the excitation light illuminates cells only for very short time (100 msec) during acquisition of each image, but was shut down before next imaging (20 sec apart). Therefore, whatever the Ca^{2+} in cytosol or in intracellular stores was measured microscopically, the photobleaching of the Ca^{2+} indicator is negligible.

Measurement of IP_3-operated Ca^{2+} release from intracellular Ca^{2+} stores in permeabilized Se^+ or Se^0 cells

The measurement of IP_3-operated Ca^{2+} release from IP_3 receptor-mediated store in the permeabilized cells was performed according to the method described by Hu et al. (2000) with minor modification. Before measurements, two intracellular-like media (ICM) with and without Mg^{2+}/ATP and a Ca^{2+}-releasing medium were freshly prepared. The Mg^{2+}/ATP-free ICM consists of 125 mM KCl, 19 mM NaCl, 10 mM HEPES, 1 mM EGTA, and 0.33 mM $CaCl_2$ (the free Ca^{2+} concentration was 50 nM), and was adjusted to pH 7.2. The complete ICM was made from Mg^{2+}/ATP-free ICM by adding 1 mM ATP and 1.4 mM $MgCl_2$ (the free Mg^{2+} concentration was 0.1 mM). The Ca^{2+}-releasing medium consists of 125 mM KCl, 19 mM NaCl, 10 mM HEPES, 1.4 mM $MgCl_2$, and 150 nM $CaCl_2$, and was also adjusted to pH 7.2. The cells plated on glass-bottomed dishes (2×10^5 cells) were incubated with 2 μM mag-fura-2/AM at 37°C for 60 min. After mag-fura-2 loading, the dishes were gently washed with HBSS and maintained at 37°C for an additional 10 min to allow for de-esterification of the Ca^{2+} indicator. The mag-fura-2-loaded cells were exposed to Mg^{2+}/ATP-free ICM for 2–3 min and then permeabilized by addition of 20 μg/ml digitonin for an extra 10 min at room temperature. Permeabilization was monitored by observing release of the cytosolic mag-fura-2 fluorescence, while the fluorescence associated with organelles persisted after permeabilization. The permeabilized cells were washed with Mg^{2+}/ATP-free ICM to remove digitonin and then exposed to complete ICM for 10 min to allow for refilling of intracellular Ca^{2+} stores. The permeabilized cells were then superfused with Ca^{2+}-releasing medium for at least 10 min. Then, the fluorescence images of the permeabilized cells excited consecutively at 340 and 380 nm were taken at emission of 510 nm before and after addition of IP_3 according to the same procedure previously described for Microscopic measurement of cytosolic Ca^{2+} concentration. The decrease of the digitized fluorescence ratio (F_{340}/F_{380}) in the permeabilized cells reflects the Ca^{2+} release from intracellular calcium stores.

Assay of Ins(1,4,5)P_3 production

The generation of IP_3 in the cells cultured in normal and selenium-supplemented medium was quantified after histamine-stimulation by the ^{3}H-IP_3 competitive binding assay (Siddiqui and English, 2000) using D-myo-IP_3 [^{3}H] assay system. The ECV304 cells (2×10^6) in HBS buffer were stimulated by histamine for 20 sec at 37°C, and then cooled down rapidly in an ice bath. The cells were lysed with ice-cold 4% perchloric acid for 20 min. The acid-insoluble component was segregated by centrifugation at 2,000*g* for 15 min at 4°C. The supernatant was neutralized to pH 7.5 by ice-cold 10M KOH. Then, $KClO_4$ in the neutralized mixture was removed by centrifugation. The neutralized supernatant was used to quantitate the IP_3 concentration according to the protocol provided by the manufacturer. All assays were performed in triplicate.

MTT assay for cell proliferation

The tetrazolium dye (MTT) assay was used to estimate the toxicity of sodium selenite to ECV304 cells (Plumb, 2004). Cells were plated in microtiter plates (96-wells) at a density of 2×10^3 cells per well and cultured in DMEM containing 10% calf serum for 24 h to achieve good attachment to the wells. One row of the wells containing only medium was used as blank. Sodium selenite of various concentrations were added in each row of wells and further cultured for 48 h. The cells were then washed with fresh medium and subjected to MTT assay. In the assay, 10 μl of MTT stock solution (10 mg/ml in PBS) was added to each well and the plates were incubated for additional 4 h at 37°C to allow MTT reduction. Thereafter, the supernatant was removed completely and 150 μl of DMSO was added to each well to solubilize the formed formazan crystals. The plate was shaken for 15 min and the optical density of each well at 595 nm was read on a Bio-Rad microplate Reader. The cell numbers in each well was expressed as the blank-subtracted optical density.

RESULTS

TrxR activity, GPx activity, and the Trx content increased in the cells supplemented with selenium

TrxR activity, GPx activity, and the Trx content in the Se^0 cells and the Se^+ cells were measured, respectively. As shown in Figure 1A, the rate of DTNB reduction by the extract from Se^+ cells was much higher than that by the extract from Se^0 cells. When 20 μM gold thioglucose, the inhibitor of TrxR, was added in both cell extracts to inhibit the activity of TrxR activity, the DTNB reduction rate became much lower and there was almost no difference between the extract from Se^+ cells and the extract from Se^0 cells in reducing DTNB. Since glutathione was removed by centrifugal filtration in preparing the crude cell extract, the existence of glutathione reductase in cell extract could not affect the DTNB reduction. At the concentration of 20 μM, gold thioglucose cannot inhibit the activity of other selenoproteins such as GPx (Gromer et al., 1998). So the reaction in the presence of gold thioglucose can be used as a blank for all of the other assays, and the measured DTNB reduction rate used as an indication of TrxR activity.

The TrxR activity expressed as DTNB reduction rate per mg protein of the cell extract and the measured Trx content in Se^+ and Se^0 cell extract is shown in Figure 1B. They increased by $72 \pm 9\%$ and $38 \pm 18\%$, respectively in the Se^+ cells in comparison with that in Se^0 cells. These results confirmed that a clear difference of TrxR activity and Trx content between the Se^+ cells and the Se^0 cells.

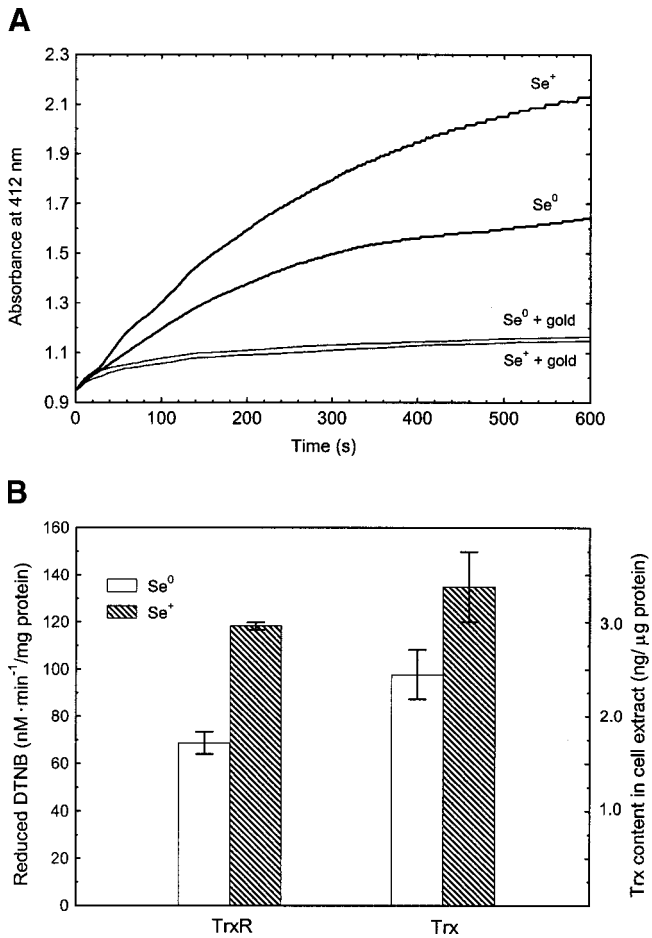

Fig. 1. Thioredoxin reductase (TrxR) activity and thioredoxin (Trx) content in Se^0 and Se^+ cells. TrxR activity in the crude cell extract was measured by the modified 5(-dithiiobis-2-nitrobenzoic acid (DTNB) reduction assay. Part A shows the DTNB reduction by the extracts from Se^+ and Se^0 cells in the absence and presence of gold thioglucose, respectively. In part B, left two columns represent the relative activities of TrxR in Se^+ and Se^0 cell extracts after correction for the DTNB reduction in the presence of gold thioglucose. The right two columns represent the Trx concentration in Se^0 cell and Se^+ cell, respectively. The experiments were done in duplicate and repeated for three times. The value represent mean ± SE for n = 3 separate experiments.

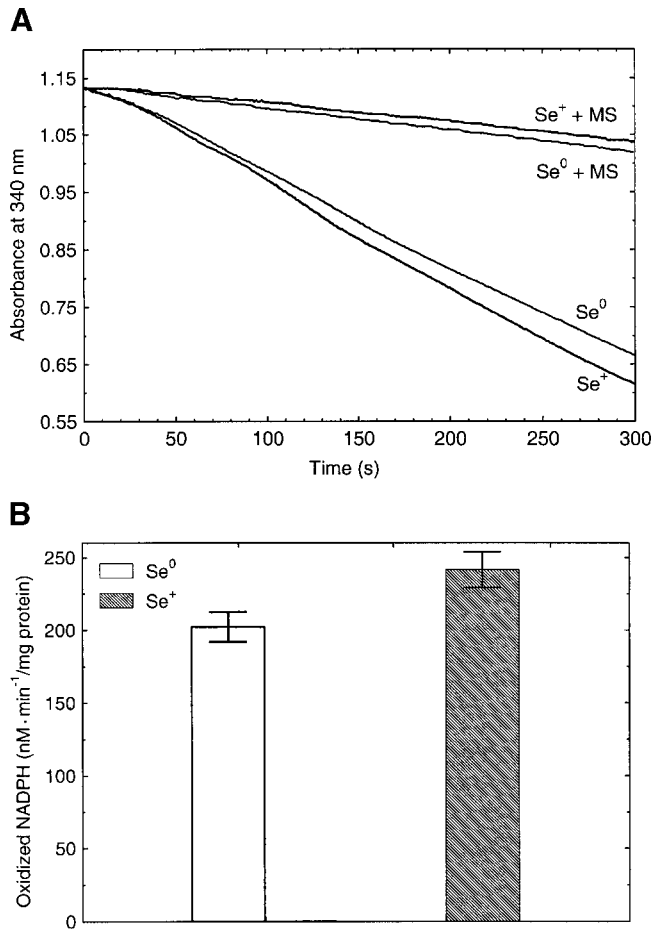

Fig. 2. GPx activity in Se^0 and Se^+ cells. Glutathione peroxidase (GPx) activity in the crude cell extract was measured by the β-nicotinamide adenine dinucleotide phosphate(NADPH) oxidation assay. Part A shows the NADPH oxidized by the extracts from Se^+ and Se^0 cells in the absence and presence of mercaptosuccinate (MS), respectively. Part B represents the activities of GPx in Se^+ and Se^0 cell extracts after correction for the NADPH oxidation in the presence of MS. The experiments were done in duplicate and repeated for three times. The value represent mean ± SE for n = 3 separate experiments.

The GPx activities expressed as the oxidation rates of the NADPH per milligram protein of the extracts from Se^+ and Se^0 cells were also measured. As shown in Figure 2A, the oxidation rate of NADPH catalyzed by the extract from Se^+ cells is faster than that by the extract from Se^0 cells. Notably, the GPx activity in Se^+ cells increased by only 20%, which is much less than the increase of TrxR in the same cells. The activity measurements of TrxR and GPx confirmed the increase of both selenoproteins in the cells cultured in selenium-supplemented medium. However, TrxR is the major selenoprotein that increased in the HUVECs cultured in selenium-supplemented medium.

Effect of selenium-supplement on cell proliferation

In the present investigation, the cells were grown in the culture medium supplemented with 1 μM sodium selenite. In order to know if the applied Na_2SeO_3 concentration is toxic to the cells, the proliferation ability of the cells grown in the medium containing various concentrations of sodium selenite ranging from 0 to 64 μM was determined by MTT assay. The results are shown in Figure 3. It was observed that below 16 μM, the presence of sodium selenite in culture medium does not inhibit but rather promotes proliferation of the cells and the concentration of sodium selenite for 50% cell survival, C_{50}, is between 16 and 32 μM. The MTT assay results may rule out any possible toxic effect of sodium selenite on the cells at the studied concentration.

Selenium-supplement reduced the IP_3 operated Ca^{2+} release from intracellular store

To find out the possible effect of selenoproteins on the sensitivity of IP_3 receptor in response to IP_3-triggered Ca^{2+} release from intracellular Ca^{2+} stores, the Se^+ and Se^0 cells were permeabilized by digitonin and exposed to D-myo-IP_3 hexasodium salt . Since fura-2 has a dissociation constant (K_d) of ~200 nM for Ca^{2+} that are close to the typical basal Ca^{2+} levels in mammalian cells (~100 nM), while the concentration of Ca^{2+} in the ER-stores is in a range of hundreds of micromoles per liter (Mogami et al., 1998), fura-2 would be completely saturated in the ER-stores and unable to provide a readout for intra-ER Ca^{2+}. Thus, mag-fura-2, which has

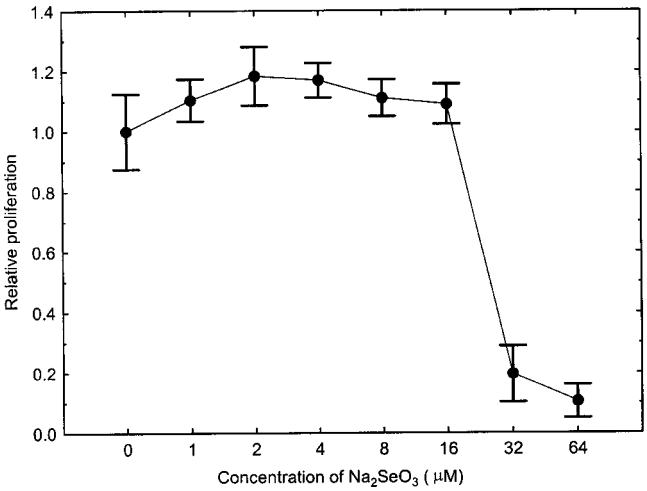

Fig. 3. Concentration-dependent effects of sodium selenite on the proliferation of ECV304 cells. The cells were pre-incubated with sodium selenite of various concentrations for 48 h before MTT assay. The relative proliferation is used to show the toxic effect of sodium selenite. Each of data is the mean of eight independent measurements, and their standard deviations (SD) are indicated as bars.

K_d of 25 μM and can avoid saturation, was chosen to measure the calcium concentration within the intracellular store.

The fluorescence images of the mag-fura-2 loaded cells in the intracellular-like medium before and after addition of digitonin are shown in Figure 4. It clearly shows that during permeabilization the mag-fura-2 cytosolic fluorescence (excited at 340 nm) started to release from the cells 2 min later, became significantly dim 5 min later, and remained unchanged 10–12 min after addition of 20 μg/ml digitonin. The remaining fluorescence of mag-fura-2 indicates the persistence of the Ca^{2+} indicator in intracellular Ca^{2+} stores.

It was observed that after refilling of Ca^{2+} stores in mag-fura-2 loaded, permeabilized Se^{+} or Se^{0} cells in the Ca^{2+} releasing medium, addition of 1 μM IP_3 in the medium caused a decrease of the fluorescence ratio of F_{340} to F_{380} associated with ER, indicating a decrease of the Ca^{2+} content in the intracellular stores. As a typical result shown in Figure 5A, the Ca^{2+} release from intracellular store induced by 1 μM IP_3 in permeabilized Se^{+} cells was significantly less than that in permeabilized Se^{0} cells. An almost unchanged fluorescence intensity of the mag-fura-2 loaded ER-stores in the permeabilized cells measured without addition of IP_3 (see the curve "no IP_3" in Fig. 5A) indicates that decrease of the IP_3-triggered fluorescence was not due to photobleaching of the calcium indicator within intracellular stores nor leakage of the indicator out of the cell. Based on three independent measurements, $20 \pm 4\%$ reduction in IP_3-triggered Ca^{2+} release from intracellular stores was observed in the cells cultured in selenium-supplemented medium (see Fig. 5B). The results demonstrate that an increase in the activities of selenoproteins significantly reduced the IP_3-triggered Ca^{2+} release from IP_3 receptor mediated intracellular stores.

Selenium-supplement increased the histamine-stimulated Ca^{2+} release and Ca^{2+} entry

Histamine, a well-established receptor agonist, was used to see how selenoproteins affect the G-protein-coupled phospholipase C-activated Ca^{2+} signaling. In the cells bathed in Ca^{2+}-free buffer, the histamine-stimulated Ca^{2+} release and subsequent Ca^{2+} entry

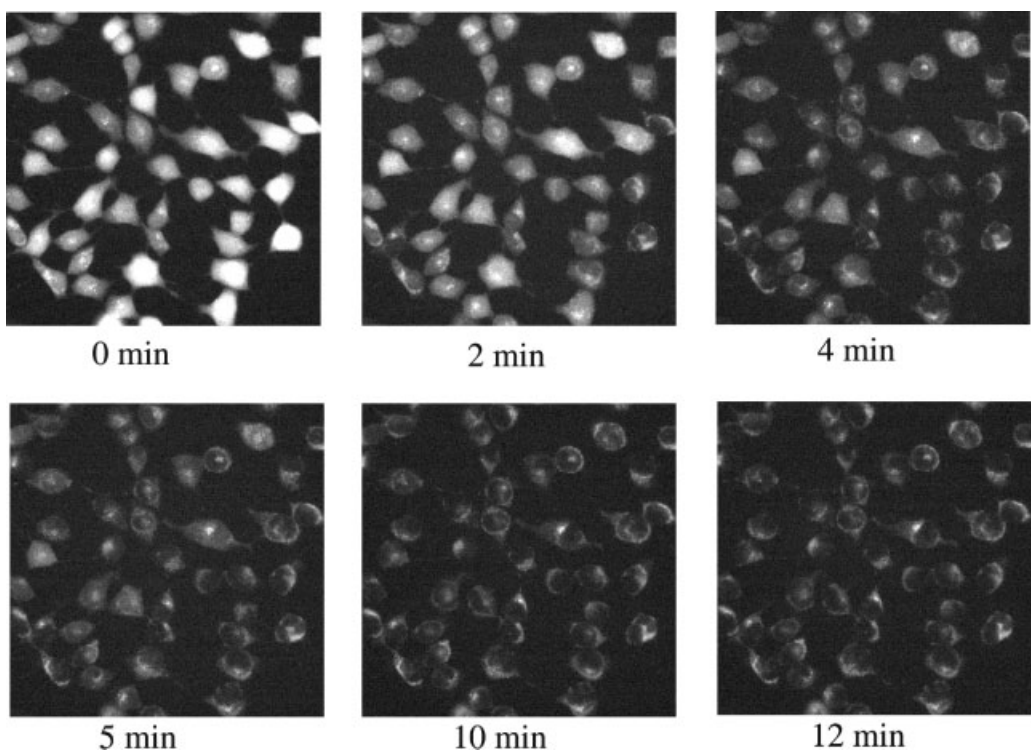

Fig. 4. The release of mag-fura-2 cytosolic fluorescence from the mag-fura-2 loaded cells during permeabilization by digitonin. The fluorescence images of the mag-fura-2 loaded cells excited at 340 nm were taken every 20 sec at emission of 510 nm after addition of 20 μg/ml digitonin in the intracellular-like medium.

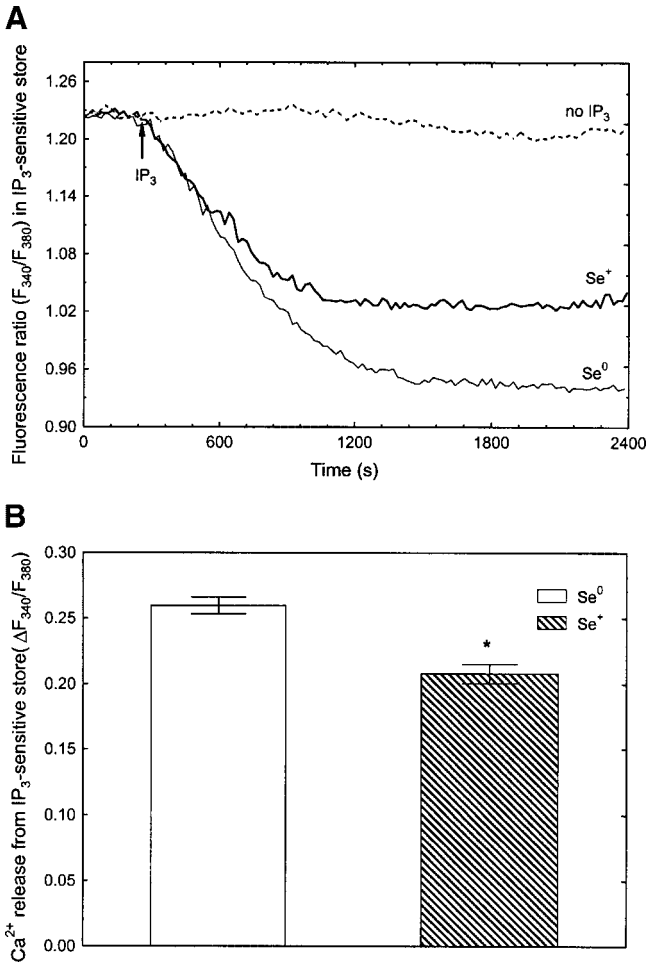

Fig. 5. Effect of selenium-supplement on the sensitivity of IP_3 receptor in response to IP_3-operated Ca^{2+} release in Se^+ and Se^0 cells. Part A: The decreased of $[Ca^{2+}]_{store}$ in Ca^{2+}-refilled intracellular stores of the permeabilized cells upon addition of 1 μM D-myo-Inositol 1,4,5-trisphosphate hexasodium salt (IP_3) are measured as the decrease of the fluorescence ratio $\Delta(F_{340}/F_{380})$ of mag-fura-2 in the stores. The curve labeled by "no IP_3" is the fluorescence ratio of mag-fura-2 in the stores without addition of IP_3. The kinetic curves are the average of those observed in six cells. Part B: The mean values of the IP_3-induced Ca^{2+} release in the permeabilized Se^+ and Se^0 cells are based on three independent measurements. SE is indicated bar. Asterisk indicates that the difference between set cells and Se^0 cells is significant with $P < 0.05$.

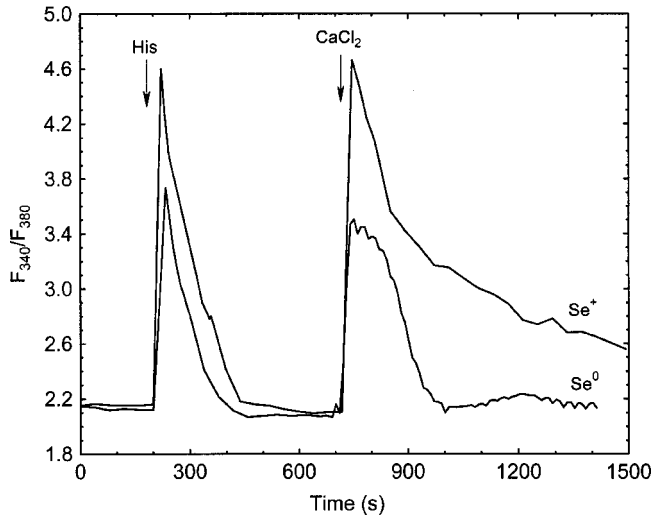

Fig. 6. Effect of selenium-supplement on the histamine-induced Ca^{2+} release and subsequent Ca^{2+} entry in the fura-2 loaded single living cells. The cytoplasmic Ca^{2+} concentration was recorded at 37°C as the ratio of the fluorescence excited at 340 nm, F_{340}, to the fluorescence excited at 380 nm, F_{380}. The measurements were carried out with Se^0 and Se^+ cells, respectively. Histamine (0.1 mM) was added first in the cell-bathed Ca^{2+}-free buffer and then 2.5 mM $CaCl_2$ was added 10 min late. All kinetic curves are the average of those observed in six cells.

initiated by addition of $CaCl_2$ are shown in Figure 6. In contrast to decrease of the IP_3-triggered Ca^{2+}release, selenium-supplement notably enhanced the histamine-induced Ca^{2+} release and the subsequent Ca^{2+} entry in the Se^+ cells. To confirm if this effect is due to enhanced sensitivity of PLC towards histamine-stimulation by selenoproteins, the IP_3 production in the histamine-stimulated cells both cultured in normal and selenium-supplemented medium were measured using ^{3}H-IP_3 competitive binding assay. As shown in Figure 7A, the histamine-stimulated production of IP_3 significantly increased in the Se^+ cells and was $102 \pm 24\%$ higher than that in Se^0 cells, which provides direct evidence that activation of PLC is enhanced by selenium-supplement. In addition, the effect of U73122, a selective PLC inhibitor that has been widely used to study the involvement of G protein-coupled PLC in receptor-mediated cell activation (Bleasdale et al., 1990; Babich et al., 1994), on Ca^{2+} signaling was also investigated in the histamine-stimulated cells. As shown in Figure 7B and Figure7C, a remarkable inhibition of either Ca^{2+} release or Ca^{2+} entry by U73122 was observed in both Se^0 and Se^+ cells, which confirms the involvement of PLC activation in histamine-induced Ca^{2+} signaling. The enhancement of histamine-induced Ca^{2+} signaling and the production of IP_3 in the Se^+ cells implies that the higher TrxR and GPx activities in cells could enhance the overall sensitivity of the histamine receptor on plasma membrane and GDP-binding protein coupled PLC activity in response to histamine-stimulation. Based on three independent measurements, $78 \pm 12\%$ enhancement of histamine-stimulated Ca^{2+} release was observed in the Se^+ cells cultured in selenium-supplemented medium (see Fig. 10).

Effect of selenium-supplement on the TG- and FCCP-induced Ca^{2+} release and Ca^{2+} entry

To further identify the internal stores from which the Ca^{2+} release could be modulated by the selenoproteins, the Ca^{2+} release induced by TG, a potent endomembrane Ca^{2+}-ATPase inhibitor, which can release Ca^{2+} from intracellular store with minimal disturbances of other signaling mechanism (Thastrup et al., 1990), and FCCP, a mitochondrial uncoupler which inhibits Ca^{2+} uptake through mitochondrial uniporter by collapsing the mitochondrial proton gradient and dissipating the mitochondrial membrane potential (Medler and Gleason, 2002), were investigated. The results are presented in Figure 8. It was found that the selenium-supplement reduced the TG-induced Ca^{2+} release and subsequent Ca^{2+} entry initiated by addition of $CaCl_2$ in Ca^{2+}-free buffer (Fig. 8A). Based on three independent measurements, the Ca^{2+} release induced by 1 μM TG was reduced by $34 \pm 8\%$ in the Se^+ cells compared with that in the Se^0 cells (also see Fig. 10). The Part B in Figure 8 shows that the selenium-supplement also markedly reduced the FCCP-induced Ca^{2+} release from mitochondria, whatever the initial sharp transient rise of the cytoplasmic Ca^{2+} or the subsequent slow phase of Ca^{2+} entry. Based on three independent measurements, $49 \pm 10\%$ reduction of FCCP-induced Ca^{2+} release from

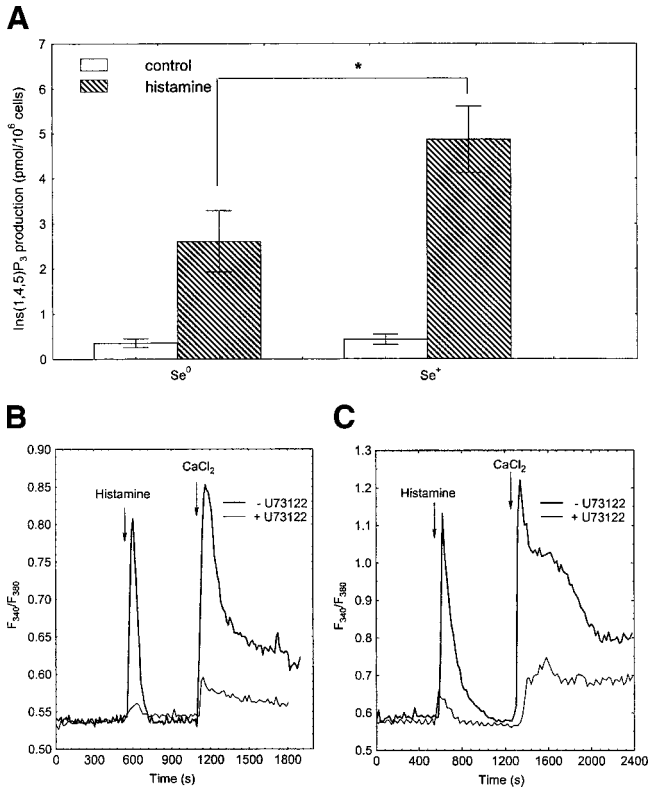

Fig. 7. IP_3 production in histamine-stimulated cells and the effect of U73122 on histamine-stimulated Ca^{2+} signal. The cytoplasmic Ca^{2+} concentration is measured as the ratio of the fluorescence excited at 340 nm, F_{340}, to the fluorescence excited at 380 nm, F_{380}, at 37°C. Part A: Production of IP_3 in Se^0 and Se^+ cell (10^6 cells/ml) without and with stimulation by 0.1 mM histamine, respectively. Each data is the mean of three independent measurements, and the SD is indicated as the bar. Part B: 0.1 mM histamine-stimulated Ca^{2+} release and subsequent Ca^{2+}-entry in the Se^0 cells pre-incubated with or without 2 μM U73122 for 15 min. The kinetic curves are the average of those observed in six cells. Part C: 0.1 mM histamine-stimulated Ca^{2+} release and subsequent Ca^{2+}-entry in the Se^+ cells pre-incubated with or without 2 μM U73122 for 15 min. The kinetic curves are the average of those observed in six cells. Asterisk indicates that the difference between set cells and Se^0 cells is significant with $P < 0.05$.

mitochondria was observed in Se^+ cells in comparison with that in the Se^0 cells. The experiment with FCCP clearly demonstrates that the selenoproteins reduce the Ca^{2+} release from mitochondria as well.

Effect of selenium-supplement on the inhibition of Ca^{2+} efflux from mitochondria by CsA

In order to know the effect of selenoproteins on the mitochondrial PTP, CsA, a well-known pharmacological PTP inhibitor which decreases the open probability of PTP (Bernardi et al., 1994; Crompton et al., 1998a), was used to treat cells and to see if selenium-supplement could have any influence on the Ca^{2+} efflux from mitochondria. As shown in Figure 9, addition of 10 μM CsA caused an immediate 5%–10% drop of the basal cytoplasmic Ca^{2+} level, indicating a reduction of Ca^{2+} efflux from mitochondria. Interestingly, the decrease of the basal $[Ca^{2+}]_i$ in the Se^+ cells was less than that in the Se^0 cells. Based on 18 cells from three independent measurements, the drop of basal $[Ca^{2+}]_i$ in the Se^+ cells was $36 \pm 10\%$ less than that in the Se^0 cells (also see Fig. 10). The larger reduction implies higher sensitivity of PTP to CsA-caused decrease of the open probability. The less reduced basal Ca^{2+} level caused by CsA in Se^+ cell may suggest that the open probability of PTP reduces in the cells expressing higher TrxR and GPx activity.

Statistical treatment of the data on the effects of selenium-supplement

The mean values and standard deviation of the change in cytoplasmic Ca^{2+} or the Ca^{2+} of intracellular store, which are represented as $\Delta(F_{340}/F_{380})$, induced by various agents are calculated based on three independent measurements. Each of the datasets obtained with Se^+ and Se^0 cells are subjected to t-test, and $P < 0.05$ was found for all cases. The statistical analysis shows that the observed IP_3-, histamine-, TG-, FCCP-induced Ca^{2+} release and CsA-caused drop of the basal cytoplasmic Ca^{2+} level in Se^+ cells significantly differ from those observed in Se^0 cells. A summary of all of these results is shown in Figure 10. It was found that the high activities of TrxR and GPx in Se^+ cells reduces the IP_3-triggered,

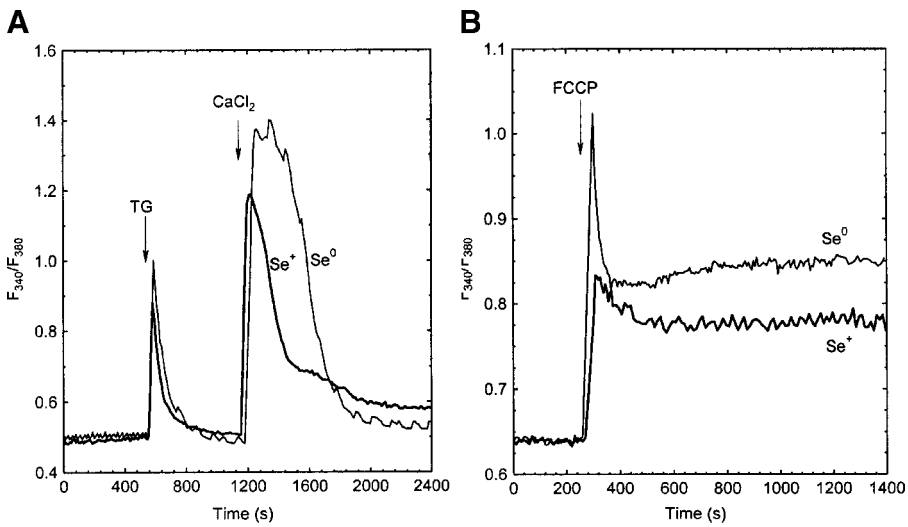

Fig. 8. Effect of selenium-supplement on the TG- and FCCP-induced Ca^{2+} signal in the fura-2 loaded single living cells bathed in the Ca^{2+}-free buffer. The cytoplasmic Ca^{2+} concentration is measured as the ratio of the fluorescence excited at 340 nm, F_{340}, to the fluorescence excited at 380 nm, F_{380}, at 37°C. The measurements were carried out with the Se^+ and Se^0 cells, respectively. Part A: Addition of 1 μM TG was followed by addition of 2.5 mM $CaCl_2$ in the cell-bathed Ca^{2+}-free buffer. Part B: 2 μM FCCP was added in the cell-bathed Ca^{2+} buffer. All kinetic curves are the average of those observed in six cells.

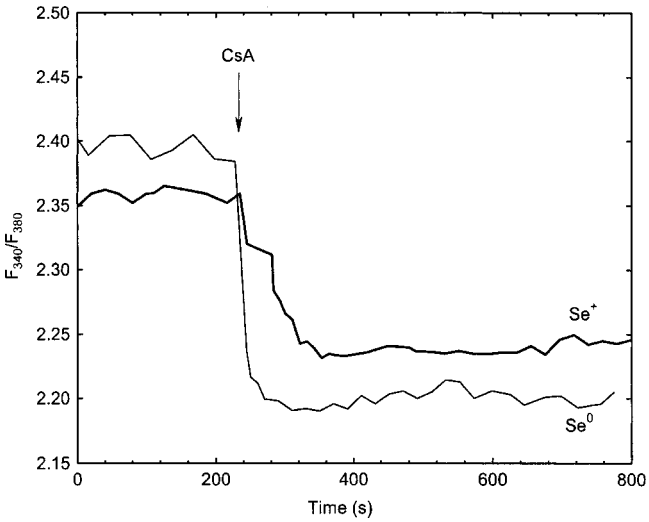

Fig. 9. Effect of selenium-supplement on the cyclosporin A (CsA)-induced lower basal intracellular Ca^{2+} level in the fura-2 loaded cells in the Ca^{2+}-buffer. The measurements were carried out with the Se^{+} and Se^{0} cells, respectively. 10 μM CsA was added in the buffer and the kinetic curves of the basal $[Ca^{2+}]_i$ change are the average of those observed in six cells.

the TG- and FCCP-induced Ca^{2+} release from intracellular stores, but enhances the histamine-stimulated Ca^{2+} release from intracellular stores. The decline of the basal cytoplasmic Ca^{2+} by CsA was found also significant in Se^{+} cells. All data suggest that the proteins, which mediate the Ca^{2+} release or uptake in ER, mitochondrial and plasma membrane, are regulated by the selenoproteins.

DISCUSSION

Selenium is known as an essential trace element involved in many physiological functions and may protect human body from cardiovascular diseases and cancer (Rayman, 2000). Most selenoproteins, in which

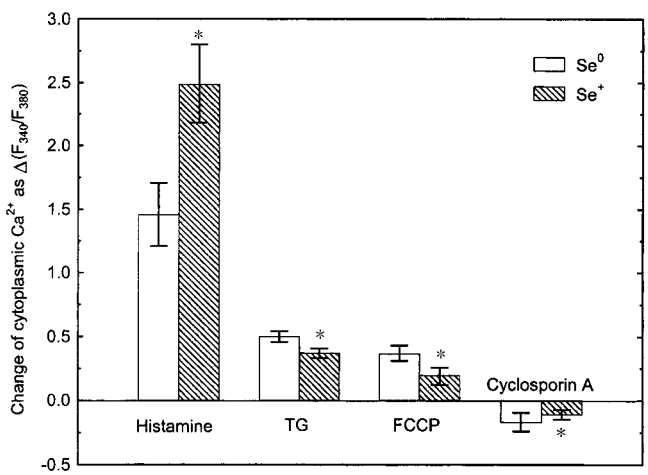

Fig. 10. Summary of the effect of selenium-supplement on the change of the cytoplasmic Ca^{2+} concentration in the fura-2 loaded Se^{+} and Se^{0} cells caused by 0.1 mM histamine, 1 μM TG, 2 μM FCCP, and 10 μM CsA, respectively. The change of cytoplasmic Ca^{2+} concentration is expressed as $\Delta(F_{340}/F_{380})$. Positive value means an increase, while negative means a decrease of the cytoplasmic Ca^{2+} level. Data are calculated based on three independent measurements and in each measurement the value is the mean of those observed from six cells. Asterisk indicates that the difference between Se^{+} cells and Se^{0} cells is significant with $P < 0.05$.

selenium is incorporated as selenocysteine, have redox function. Redox regulation has become an increasingly interesting area, since many molecular and cellular functions such as transcription, translation, enzymatic activity, cell proliferation, and apoptosis are regulated in this way. Selenoproteins such as TrxR and GPx are particularly important for redox regulation of protein function and signaling via thiol-redox control (Biguet et al., 1994; Arnér and Holmgren, 2000). The events upstream calcium signaling involve a variety of proteins on plasma and cytoplasmic membranes such as receptors and ion channels on cell surface, G-protein-coupled phospholipase C family, IP_3 or ryanodine receptor on ER or SR membrane, Ca^{2+}-ATPase on both plasma and ER membranes, and the PTP on mitochondrial inner membrane. They all contain thiols and are potential targets for redox regulation by cellular TrxR/Trx system (see equations below) as well as by glutathione/GPx system. It has been reported that without selenium-supplement the normal culture medium, which contains 10% fetal-bovine serum, has a selenium concentration less than 0.01 μM (Gallegos et al., 1997). In the present work, the cells were cultured in the medium containing 1 μM selenium for expressing more selenoproteins. Based on the data from literature (Thomson and Paterson, 2001), the serum selenium concentration in the persons, who have a selenium intake of around 60 μg/day in diet, is about 1 μM. Comparing various stimuli-induced Ca^{2+} release from all possible internal stores in the cells cultured in selenium-supplemented medium with that in the cells cultured in normal medium may provide a better evaluation on the role of selenoproteins, particularly TrxR and GPx, in regulating Ca^{2+} signal.

The IP_3-operated Ca^{2+} channel is responsible for the agonist-stimulated release of Ca^{2+} from IP_3-sensitive ER. The competitive binding study of radiolabeled IP_3 has suggested that the IP_3 receptor is a putative target for different regulatory mechanisms including phosphorylation by protein kinase A and C or calmodulin kinase (Ferris et al., 1991). We permeabilized the cells cultured in either selenium-supplemented or normal medium, and used exogenous IP_3 to directly probe the sensitivity of IP_3 receptor in IP_3-triggered Ca^{2+} release from intracellular stores. Our data revealed that the sensitivity of IP_3 receptor in the cells expressing higher selenoprotein activity was significantly reduced. This may attribute to the reduction of critical disulfide sites on IP_3 receptor to sulfhydryl. Recently, Uchida et al. (2003) identified two regions of the IP_3R, residues 1–223 and 651–1,130, are critical for IP_3-induced gating as well as a highly conserved cysteine residue at position 2,613 located within the C-terminal tail as being essential for channel opening. It may suggest that the numbers of sulfhydryl in those critical regions of IP_3 receptor depend on the activity of TrxR and Trx in the following manner.

$$\text{Trx-S}_2 + \text{NADPH} + \text{H}^+ \overset{\text{TrxR}}{\longleftrightarrow} \text{Trx-(SH)}_2 + \text{NADP}^+ \quad (1)$$

$$\text{Trx-(SH)}_2 + \text{Protein-S}_2 \longleftrightarrow \text{Trx-S}_2 + \text{Protein-(SH)}_2 \quad (2)$$

Although, GPx cannot directly reduce the disulfides of proteins to thiols, it may also play a role for maintaining cellular thiol homeostasis by eliminating hydroperoxides of lipids and H_2O_2 in cells. Since Trx is able to directly reduce the protein's disulfides and 70% increase of TrxR activity but only 20% increase of GPx activity

were found in the cells cultured in selenium-supplemented medium, it may be speculated that the increased TrxR activity would be mainly responsible for a more reduced form of IP_3 receptors in the cells expressing more selenoproteins.

Recently, Hu et al. (2000) proved that the NADPH oxidase-derived H_2O_2 increases the sensitivity of intracellular Ca^{2+} stores to IP_3 in human endothelial cells. Now, our data suggest that higher activity of selenoproteins especially TrxR decrease its sensitivity to IP_3. Both our finding and the report by Hu et al. seem to draw a complete picture that the sensitivity of IP_3 receptor in response to IP_3-operated Ca^{2+} release could be very well regulated by cellular redox status.

The effect of selenium-supplement on histamine-stimulated Ca^{2+} release and subsequent Ca^{2+} entry is totally different from that observed in the IP_3-stimulation. An enhancement of histamine-induced Ca^{2+} release and subsequent Ca^{2+} entry are found in the Se^+ cells. The histamine-stimulated cytoplasmic Ca^{2+} mobilization is a typical receptor agonist-induced Ca^{2+} signaling in which GDP-binding protein mediates activation of PLC and leads to a generation of two second messengers: IP_3 and diacylglycerol. IP_3 then induces the release of Ca^{2+} from intracellular stores. Since a reduction of the IP_3 receptor sensitivity to IP_3-stimulated Ca^{2+} release was found in the Se^+ cells, there must be even greater increase of IP_3 generation in the histamine-stimulated Se^+ cells to account for the enhancement of Ca^{2+} release from intracellular stores. Our result confirmed the speculation. The production of IP_3 induced by histamine in Se^+ cells is much greater than the production in Se^0 cells. Regulation of four mammalian PLC isozymes ($\beta1-\beta4$) has been extensively studied (Rhee and Bae, 1997). Stimulation of PLC by many agonists (e.g. histamine, vasopressin, α- and β-adrenergic agonists) occurs through receptors coupled to heterotrimeric G-proteins and is mediated by the α subunit members of the Gq subfamily and by $\beta\gamma$ subunits. The increase of IP_3 production in Se^+ cells could be the result of an increase of the G-protein coupled PLC activity and/or increased histamine receptor sensitivity to its agonist in the cells expressing more selenoproteins. The increased activity of PLC may be related to the redox status of PLC itself, the coupled G-protein or the agonist receptor. It has been reported that the thiol agent, *p*-chloromercuribenzene sulfonic acid (pCMBS), inactivates the $P2Y_{12}$ receptor by interacting with two free cysteines in its extracellular domains (Ding et al., 2003). Another thiol reagent (*N*-ethylmaleimide) reduced Sambucus nigra agglutinin-induced lysozyme release from neutrophils through the mechanisms involving G-proteins and the thiol-dependent signaling systems (Gorudko and Timoshenko, 2000). Inhibition of protein activity by thiol reagents is indicative of the participation of cysteine residues in biochemical events. Thus, it could be expected that the higher activity of selenoproteins in the Se^+ cell may result in higher sensitivity of agonist receptor, G-protein and PLC activity by switching these proteins to a more reduced form. Either the reduction of IP_3-triggered Ca^{2+} release or the enhancement of histamine-stimulated Ca^{2+} release in the Se^+ cells leads to same conclusion that selenoproteins affect Ca^{2+} signaling through redox regulation of the proteins involved in the pathways upstream Ca^{2+} release from intracellular Ca^{2+} stores.

The effects of selenium-supplement on TG- and FCCP-induced cytoplasmic Ca^{2+} signal (see Fig. 8) indicate not only the existence of TG-and FCCP-sensitive Ca^{2+} stores, but also the redox-regulation of the Ca^{2+} release or efflux from those stores. The reduction of the TG-induced Ca^{2+} release and the subsequent Ca^{2+} entry in Se^+ cells may suggest that the higher selenoprotein activity makes the Ca^{2+}-ATPase on ER membrane less sensitive to its inhibitor by thiol-redox regulation. FCCP can dissipate the membrane potential across the mitochondrial inner membrane that normally sustains the activity of uniporter and elevation of cytoplasmic Ca^{2+} level (Medler and Gleason, 2002). The reduced elevation of the cytoplasmic Ca^{2+} level by FCCP in Se^+ cells suggests that the mitochondrial uniporter is also a target protein for the thiol-redox regulation by selenoproteins. Besides the uniporter-dependent Ca^{2+} release from uncoupled mitochondria, the PTP is another important protein for mediating Ca^{2+} efflux from mitochondria. It consists of three core components, the voltage-dependent anion channel of the outer mitochondrial membrane, the adenine nucleotide translocase of the inner mitochondrial membrane, and cyclophilin-D in the mitochondrial matrix (Halestrap et al., 1998; Crompton, 1999). The immunosuppressant CsA can selectively bind to cyclophilin D and displace it from its binding site in PTP, which favors the closed state of the pore (Crompton et al., 1998b). The CsA-induced drop of the basal cytoplasmic Ca^{2+} level observed by us and others (Smaili et al., 2001) is the result of lower Ca^{2+} efflux from mitochondria via PTP. The smaller drop of the basal cytoplasmic Ca^{2+} level in Se^+ cells implies that a redox-alteration of PTP by selenoproteins occurs.

TrxR is the predominant selenoprotein comprising 43% of the total selenoproteins in HUVECs (Anema et al., 1999). Working with its substrate, Trx, and the cofactor NADPH, TrxR forms a powerful dithiol-disulphide oxidoreductase system that has multiple roles. The reduced Trx is able to reduce a variety of biological molecules such as thioredoxin peroxidase, ribonucleotide reductase, and even oxidized glutathione (Nordberg and Arner, 2001). The effect of selenium-supplement on intracellular Ca^{2+} signaling presented by this study is likely to be mediated by the TrxR/Trx system, though other selenoproteins such as GPx may also have some contribution. Since the intracellular calcium signaling controls many cellular functions, understanding the regulation of intracellular Ca^{2+} signaling by selenoproteins such as TrxR and GPx is certainly a necessary step for understanding the regulation of various cell functions by the selenoproteins.

ACKNOWLEDGMENTS

L. Zhong was supported by the fellowships from Hagelén Foundation and Wang Kung Cheng Foundation.

LITERATURE CITED

Adler V, Pincus MR, Posner S, Upadhyaya P, El-Bbayoumy K, Ronai Z. 1996. Effects of chemopreventive selenium compounds on Jun N-kinase activities. Carcinogenesis 17:1849–1854.

Anema SM, Walker SW, Howie AF, Arthur JR, Nicol F, Beckett GJ. 1999. Thioredoxin reductase is the major selenoprotein expressed in human umbilical-vein endothelial cells and is regulated by protein kinase C. Biochem J 342:111–117.

Arnér ES, Holmgren A. 2000. Physiological functions of thioredoxin and thioredoxin reductase. Eur J Biochem 267:6102–6109.

Babich M, Alford GE, Nissenson RA. 1994. A novel phospholipase C inhibitor and phorbol esters reveal selective regulation of thrombin- and parathyroid hormone-stimulated signaling pathways in rat osteosarcoma cells. J Pharmacol Exp Ther 269:172–177.

Bernardi P, Broekemeyer KM, Pfeiffer DR. 1994. Recent progress on regulation of the mitochondrial permeability transition pore: A cyclosporin-sensitive pore in the inner mitochondrial membrane. J Bioenerg Biomembr 26:509–517.

Berridge MJ, Bootman MD, Lipp P. 1998. Calcium—a life and death signal. Nature 395:645–658.
Berridge MJ, Lipp P, Bootman MD. 2000. The calcium entry pas de deux. Science 287:1604–1605.
Berry MJ, Banu L, Chen YY, Mandel SJ, Kieffer JD, Harney JW, Larsen PR. 1991. Recognition of UGA as a selenocysteine codon in type I deiodinase requires sequences in the 3′ untranslated region. Nature 353:273–276.
Biguet C, Wakasugi N, Mishal Z, Holmgren A, Chouaib S, Tursz T, Wakasugi H. 1994. Thioredoxin increases the proliferation of human B-cell lines through a protein kinase C-dependent mechanism. J Biol Chem 269:28865–28870.
Bleasdale JE, Thakur NR, Gremban RS, Bundy GL, Fitzpatrick FA, Smith RJ, Bunting S. 1990. Selective inhibition of receptor-coupled phospholipase C-dependent processes in human platelets and polymorphonuclear neutrophils. J Pharmacol Exp Ther 255:756–768.
Chaudiere J, Wilhelmsen EC, Tappel AL. 1984. Mechanism of selenium-glutathione peroxidase and its inhibition by mercaptocarboxylic acids and other mercaptans. J Biol Chem 259:1043–1050.
Crompton M. 1999. The mitochondrial permeability transition pore and its role in cell death. Biochem J 341:234–249.
Crompton M, Ellinger H, Costi A. 1998a. Inhibition by cyclosporin A of a Ca^{2+}-dependent pore in heart mitochondria activated by inorganic phosphate and oxidative stress. Biochem J 255:357–360.
Crompton M, Virji S, Ward JM. 1998b. Cyclophilin-D binds strongly to complexes of the voltage-dependent anion channel and the adenine nucleotide translocase to form the permeability transition pore. Eur J Biochem 258:729–735.
Ding Z, Kim S, Dorsam RT, Jin J, Kunapuli SP. 2003. Inactivation of the human $P2Y_{12}$ receptor by thiol reagents requires interaction with both extracellular cysteine residues. Cys17 and Cys270. Blood 101:3908–3914.
Ferris CD, Huganir RL, Bredt DS, Cameron AM, Snyder SH. 1991. Inositol trisphosphate receptor: Phosphorylation by protein kinase C and calcium calmodulin-dependent protein kinases in reconstituted lipid vesicles. Proc Natl Acad Sci 88:2232–2235.
Gallegos A, Berggren M, Gasdaska JR, Powis G. 1997. Mechanisms of the regulation of thioredoxin reductase activity in cancer cells by the chemopreventive agent selenium. Cancer Res 57:4965–4970.
Ganther HE. 1999. Selenium metabolism, selenoproteins and mechanisms of cancer prevention: Complexities with thioredoxin reductase. Carcinogenesis 20:1657–1666.
Gorudko IV, Timoshenko AV. 2000. Effect of signaling inhibitors on the release of lysozyme from human neutrophils activated by Sambucus nigra agglutinin. Biochemistry (Mosc) 65:940–945.
Gromer S, Arscott LD, Williams CHJ, Schirmer RH, Becker K. 1998. Human placenta thioredoxin reductase. Isolation of the selenoenzyme, steady state kinetics, and inhibition by therapeutic gold compounds. J Biol Chem 273:20096–20101.
Halestrap AP, Kerr PM, Javadov S, Woodfield K. 1998. Elucidating the molecular mechanism of the permeability transition pore and its role in reperfusion injury of the heart. Biochim Biophys Acta 1366:79–94.
Helmy MH, Ismail SS, Fayed H, El-Bassiouni EA. 2000. Effect of selenium supplementation on the activities of glutathione metabolizing enzymes in human hepatoma Hep G2 cell line. Toxicology 144:57–61.
Hill KE, McCollum GW, Burk RF. 1997. Determination of thioredoxin reductase activity in rat liver supernatant. Anal Biochem 253:123–125.
Hu Q, Zheng G, Zweier JL, Deshpande S, Irani K, Ziegelstein RC. 2000. NADPH oxidase activation increases the sensitivity of intracellular Ca^{2+} stores to inositol 1,4,5-trisphosphate in human endothelial cells. J Biol Chem 275: 15749–15757.
Kidd JF, Pilkington MF, Schell MJ, Forgarty KE, Skepper JN, Taylor CW, Thorn P. 2002. Paclitaxel affects cytosolic calcium signals by opening the mitochondrial permeability transition pore. J Biol Chem 277:6504–6510.
Landolfi B, Curci S, Debellis L, Pozzan T, Aldebaran M, Hofer AM. 1998. Ca^{2+} homeostasis in the agonist-sensitive internal store: Functional interactions between mitochondria and the ER measured in situ in intact cells. J Cell Biol 142:1235–1243.
Lewin MH, Arthur JR, Riemersma RA, Nicol F, Walker SW, Millar EM, Howie AF, Beckett GJ. 2002. Selenium supplementation acting through the induction of thioredoxin reductase and glutathione peroxidase protects the human endothelial cell line EAhy926 from damage by lipid hydroperoxides. Biochim Biophys Acta 1593:85–92.
Lytton J, Westlin M, Hanley MR. 1991. Thapsigargin inhibits the sarcoplasmic or endoplasmic reticulum Ca^{2+}- ATPase family of calcium pumps. J Biol Chem 266:17067–17071.
Makino Y, Yoshikawa N, Okamoto K, Hirota K, Yodoi J, Makino I, Tanaka H. 1999. Direct association with thioredoxin allows redox regulation of glucocorticoid receptor function. J Biol Chem 274:3182–3188.
Makropoulos V, Bruning T, Schulze-Osthoff K. 1996. Selenium-mediated inhibition of transcription factor NF-kappa B and HIV-1 LTR promoter activity. Arch Toxicol 70:277–283.
Medler K, Gleason EL. 2002. Mitochondrial Ca^{2+} buffering regulates synaptic transmission between retinal amacrine cells. J Neurophysiol 87:1426–1439.
Miller S, Walker SW, Arthur JR, Lewin MH, Pickard K, Nicol F, Howie AF, Beckett GJ. 2002. Selenoprotein expression in endothelial cells from different human vasculature and species. Biochim Biophys Acta 1588:85–93.
Mogami H, Tepikin AV, Petersen OH. 1998. Termination of cytosolic Ca^{2+} signals: Ca^{2+} reuptake into intracellular stores is regulated by the free Ca^{2+} concentration in the store lumen. EMBO J 17:435–442.
Murchison D, Griffith WH. 2000. Mitochondria buffer non-toxic calcium loads and release calcium through the mitochondrial permeability transition pore and sodium calcium exchanger in rat basal forebrain neurons. Brain Res 854:139–151.
Nordberg J, Arner ESJ. 2001. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. Free Radic Biol Med 31:1287–1312.
Paglia ED, Valentine WN. 1979. Studies on the quantitative and qualitative characterization of erythrocytes glutathion peroxidase. J Lab Clin Med 70:158–169.
Pinton P, Pozzan T, Rizzuto R. 1998. The Golgi apparatus is an inositol 1,4,5-trisphosphate-sensitive Ca^{2+} store, with functional properties distinct from those of the endoplasmic reticulum. EMBO J 17:5298–5308.
Plumb JA. 2004. Cell sensitivity assay: The MTT assay. Methods Mol Med 88:165–169.
Putney JWJ, Broad LM, Braun FJ, Lievremont JP, Bird GS. 2001. Mechanisms of capacitative calcium entry. J Cell Sci 114:2223–2229.
Rayman MP. 2000. The importance of selenium to human health. Lancet 356 :233–241.
Rhee SG, Bae YS. 1997. Regulation of phosphoinositide-specific phospholipase C isozymes. J Biol Chem 272:15045–15048.
Rizzuto R, Bastianutto C, Brini M, Murgia M, Pozzan T. 1994. Mitochondrial Ca^{2+} homeostasis in intact cells. J Cell Biol 126:1183–1194.
Sauer H, Wartenberg M, Hescheler J. 2001. Reactive oxygen species as intracellular messengers during cell growth and differentiation. Cell Physiol Biochem 11:173–186.
Schieke SM, Brivita K, Klotz LO, Sies H. 1999. Activation pattern of mitogen-activated protein kinases elicited by peroxynitrite: Attenuation by selenite supplementation. FEBS Lett 448:301–303.
Siddiqui RA, English D. 2000. Phosphatidylinositol 3′-kinase-mediated calcium mobilization regulates chemotaxis in phosphatidic acid-stimulated human neutrophils. Biochim Biophys Acta 1483:161–173.
Smaili SS, Stellato KA, Burnett P, Thomas AP. 2001. Cyclosporin A inhibits inositol 1,4,5-trisphosphate-dependent Ca^{2+} signals by enhancing Ca^{2+} uptake into the endoplasmic reticulum and mitochondria. J Biol Chem 276:23329–23340.
Spector A, Yan G-Z, Huang RR, McDermott MJ, Gascoyne PR, Pigiet V. 1988. The effect of H_2O_2 upon thioredoxin-enriched epithelial cells. J Biol Chem 263: 4984–4990.
Stapleton SR, Garlock GL, Foellmi-Adams L, Kletzien RF. 1997. Selenium: Potent stimulator of tyrosyl phosphorylation and activator of MAP kinase. Biochim Biophys Acta 1355:259–269.
Stewart MS, Spallholz JE, Neldner KH, Pence BC. 1999. Selenium compounds have disparate abilities to impose oxidative stress and induce apoptosis. Free Radic Biol Med 26:42–48.
Thastrup O, Cullen PJ, Drobak BK, Hanley MR, Dawson AP. 1990. Thapsigargin, a tumor promoter, discharges intracellular Ca^{2+} stores by specific inhibition of the endoplasmic reticulum Ca^{2+} ATPase. Proc Natl Acad Sci 87:2466–2470.
Thomson CD, Paterson E. 2001. Australian and New Zealand nutrient reference values for selenium. Wellington: Ministry of Health. 40p.
Tolando R, Jovanovic A, Brigelius-Flohe R, Ursini F, Maiorino M. 2000. Reactive oxygen species and proinflammatory cytokine signaling in endothelial cells: Effect of selenium supplementation. Free Radic Biol Med 28:979–986.
Uchida K, Miyauchi H, Furuichi T, Michikawa T, Mikoshiba K. 2003. Critical regions for activation gating of the inositol 1,4,5-trisphosphate receptor. J Biol Chem 278:16551–16560.
Van Baelen K, Vanoevelen J, Missiaen L, Raeymaekers L, Wuytack F. 2001. The Golgi PMR1 P-type ATPase of caenorhabditis elegans: Identification of the gene and demonstration of calcium and manganese transport. J Biol Chem 276: 10683–10691.
Wright SK, Viola RE. 1998. Evaluation of methods for the quantitation of cysteines in proteins. Anal Biochem 265:8–14.
Yang G, Chen J, Wen Z, Ge K, Zhu L, Chen X, Chen X. 1984. The role of selenium in Keshan disease. Adv Nutr Res 6:203–231.