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Characterization of Store-operated Ca^{2+} Entry in 3T3-L1 Cells

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ABSTRACT

3T3-L1 preadipocytes differentiate into mature adipocytes and are useful for studying the mechanism of obesity. The mobilization of intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) is related to adipocyte differentiation. Therefore, we studied the mechanisms regulating $[Ca^{2+}]i$ mobilization in isolated fura-2-loaded 3T3-L1 preadipocytes. Thapsigargin and prostaglandin F2α (PGF2α) are known to cause Ca^{2+} influx from the extracellular space. However, this influx was inhibited by 2 store-operated Ca^{2+} entry (SOCE) inhibitors : 2-aminoethoxydiphenyl borate and Ni^{2+}. These results indicated the presence of a SOCE system in 3T3-L1 preadipocytes. Although the actin cytoskeleton plays an important role in many cell functions, whether the actin network affects SOCE remains controversial. Cytochalasin D, an actin-depolymerizing agent, and calyculin A, a cortical actin-reorganizing agent, did not inhibit thapsigargin-induced $[Ca^{2+}]i$ mobilization. These results suggested that the actin cytoskeleton does not participate in SOCE in 3T3-L1 preadipocytes. Canonical transient receptor potential channels (TRPs) have recently been reported to be involved in SOCE. We detected TRPC1 and TRPC2 messenger RNAs by reverse transcriptase polymerase chain reaction. However, we did not observe Sr^{2+} influx. Therefore, we inferred that TRPCs do not take part in SOCE in 3T3-L1 preadipocytes.

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Key words : calcium, store-operated Ca^{2+} entry, cytoskeleton, preadipocyte

Introduction

Obesity is a major risk factor for hypertension, heart diseases, and diabetes. Furthermore, increasing adipose tissue results in obesity. Therefore, the adipogenic mechanism of preadipocytes has been extensively studied. In many studies, 3T3-L1 preadipocytes, which differentiate into adipocytes under appropriate conditions, are often used as an *in vitro* model.

Ca^{2+} is an important regulator of various intracellular events. Intracellular mobilization of Ca^{2+} controls many cellular functions, including proliferation, transcription, differentiation, and apoptosis[1]. The process of adipocyte differentiation is affected by the mobilization of intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) in 3T3-L1 preadipocytes.

Nakada et al. have reported that prostaglandin F2α (PGF2α) increases intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) in 3T3-L1 preadipocytes[2]. There are PGF2α receptors (FP receptors) in 3T3-L1 preadipocytes[3]. However, the precise system involved in cytosolic Ca^{2+} mobilization in 3T3-L1 cells remains unclear. Ca^{2+} enters the cells from the extracellular

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space through various types of Ca^{2+} entry systems (i.e., voltage-operated Ca^{2+} channel, receptor-operated Ca^{2+} channel, and store-operated Ca^{2+} entry [SOCE])[4]. Therefore, we investigated the Ca^{2+} entry system that uses PGF2α and how the ion is transported into the 3T3-L1 cells.

MATERIALS AND METHODS

1. Cell culture and adipocyte differentiation

3T3-L1 preadipocytes were obtained from the American Type Culture Collection (Manassas, VA, USA) and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% calf serum, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37°C in a humidified atmosphere with 5% CO_2.

Two days after the cells reached confluence (day 0), adipocyte conversion was induced with 0.25 μM dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine, and 1 μg/mL insulin in DMEM supplemented with 10% fetal calf serum. After 2 days (day 2), the culture medium was replaced with DMEM supplemented with 10% fetal calf serum and 1 μg/mL insulin. The medium was changed every other day with DMEM supplemented with 10% fetal calf serum. After 10 days (day 10), 3T3-L1 preadipocytes differentiated into adipocytes (3T3-L1 adipocytes). Adipocyte differentiation was confirmed by Oil Red O staining.

2. Measurement of $[Ca^{2+}]i$

Isolated 3T3-L1 cells were loaded with 2 μM fura-2/acetoxymethyl ester (fura-2/AM) in Krebs-Ringer HEPES buffer (123 mM NaCl, 4.6 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 1.2 mM $CaCl_2$, 11.1 mM glucose, 0.1% bovine serum albumin, and 10 mM HEPES, pH 7.4) for 30 minutes at 37°C. The cells were centrifuged and resuspended in the above buffer and incubated for further 30 minutes at 37°C to allow hydrolysis of the intracellular fura-2 ester. Fluorescence of the fura-2 loaded cells was monitored using a fluorescence spectrophotometer (F-2000, Hitachi Medical Corporation, Tokyo, Japan) at 25°C with continuous stirring at an emission wavelength of 510 nm and excitation wavelengths of 340 and 380 nm. The $[Ca^{2+}]$i was indicated by the ratio of the fluorescence intensity at the 340-nm excitation wavelength to that at 380 nm (I340/I380)[5].

3. Fluorescent staining of filamentous actin

Fluorescent staining of filamentous actin (F-actin) was monitored as previously described[6]. Briefly, 3T3-L1 preadipocytes cultured on a coverslip were fixed in 10% formaldehyde at room temperature. The fixed cells were permeabilized with 0.1% Triton-X, followed by incubation with rhodamine-phalloidin. Fluorescent images were observed with a microscope (Microphoto-FX, Nikon Corp., Tokyo, Japan) and photographed (Kodak Tri-X Pan film, Eastman Kodak Co., Rochester, NY, USA).

4. Reverse transcriptase polymerase chain reaction for canonical transient receptor potential proteins

The reverse transcription (RT) reaction was performed with the SuperScript II 1st Strand DNA Synthesis Kit (Invitrogen Corp., Carlsbad, CA, USA) followed by 50 cycles of polymerase chain reaction (PCR) (1 minute at 94°C, 1 minute at 54.4°C, and 2 minutes at 72°C). Specific primers for canonical transient receptor potential channels (TRPs)[1-7] were as follows[7,8]: murine (m) TRPC1, forward 5′-GATTTTGGGAAATTTCTGGG-3′, reverse 5′-TGTTATCAGCTGGAAGCT-3′; mTRPC2, forward 5′-GACATGATCCGGTTCATG-3′, reverse 5′-CTGGATCTTCTGGAAGGA-3′; mTRPC3, forward 5′-GACATATTCAAGTTCATGGT-3′; reverse, 5′-CTGGATCTCTTGGTATGA-3′; mTRPC4, forward 5′-TGGGACATGTGGCACCCCAC-3′, reverse 5′-ACGTGGAAAACGCGTTGTTCTG-3′; mTRPC5, forward 5′-GACTAGTCTTGATATACTCAAATTTCTC-3′, reverse 5′-GGGGTACCTCAGCATGATGGGCAATG-3′; mTRPC6, forward 5′-GATATCTTCAAATTCATGGTC-3′, reverse 5′-CTCAATTTCCTGGAATGAAC-3′; mTRPC7, forward 5′-TGTAACGCTGCACAACGTCTCA-3′, reverse 5′-AATTCCTCATGCCAGCCTGGTA-3′. The expected lengths of amplified fragments were 356, 297, 309, 304, 365, 309, and 767 base pairs for mTRPC1, mTRPC2, mTRPC3, mTRPC4, mTRPC5, mTRPC6, and mTRPC7, respectively[7,8].

5. Statistical analysis

Statistical analysis was performed by analysis of variance. The level of statistical significance was set at $P<0.05$.

6. Reagents

The reagents were purchased from the following companies: fura-2/AM from Dojindo Laboratories (Kumamoto, Japan); thapsigargin, PGF2α, 3-isobutyl-1-methylxanthine, insulin, dexamethasone, cytochalasin D, and calyculin A from Sigma-Aldrich Co., (St. Louis, MO, USA); 2-aminoethoxydiphenyl borate (2-APB) from Tokyo Chemical Industry Co., (Tokyo, Japan); and DMEM from Invitrogen Co., (Carlsbad, CA, USA). All the other chemicals were of reagent grade.

Results

1. Effects of PGF2α and thapsigargin on [Ca^{2+}]i mobilization in 3T3-L1 preadipocytes

PGF2α (1 μM) brought about a two-phase increase of [Ca^{2+}]i in the presence of extracellular Ca^{2+}. The first phase was rapid and transient, and the second phase was a sustained rise (Fig. 1A (a)). However, in the absence of extracellular Ca^{2+}, the phase was significantly reduced (Fig. 1A (a)). In the absence of extracellular Ca^{2+}, the difference between the ratios at 600 sec and at the beginning of the determination was significantly lower in PGF2α-treated cells than in control cells ($P<0.001$) (Fig. 1A (b)). PGF2α binds to FP receptor-coupled Gq protein and activates phospholipase C, resulting in inositol 1, 4,5-trisphosphate (IP3)-stimulated Ca^{2+} release from the endoplasmic reticulum (ER) to deplete luminal Ca^{2+} [9,10]. The depletion of luminal Ca^{2+} in the ER

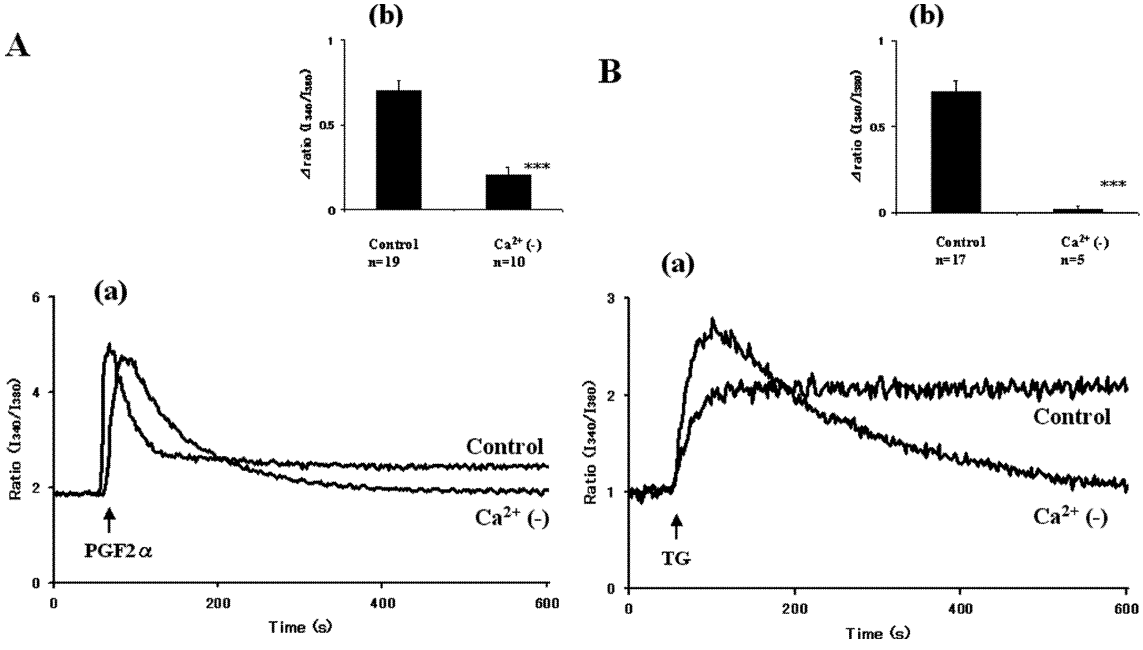

Fig. 1. Effects of PGF2α and thapsigargin on [Ca^{2+}]i mobilization in 3T3-L1 preadipocytes. PGF2α (1 μM) and thapsigargin (2 μM) were added 50 seconds after the beginning of fluorescence determination in the presence or absence of extracellular Ca^{2+} (1.2 mM). A (a): Effect of PGF2α on [Ca^{2+}]i mobilization in 3T3-L1 preadipocytes. Typical tracings are shown. A (b): The difference between the ratios of I340/I380 at 600 seconds and at the beginning of fluorescence determination in the presence and absence of extracellular Ca^{2+}. B (a): Effect of thapsigargin on [Ca^{2+}]i mobilization in 3T3-L1 preadipocytes. Typical tracings are shown. B (b): The difference between the ratios of I340/I380 at 600 seconds and at the beginning of fluorescence determination in the presence and absence of extracellular Ca^{2+}. Each value represents the mean$\pm$S.E.; ***, $P<0.001$.

triggers Ca^{2+} influx from the extracellular space. To examine this possibility, we investigated the effect of thapsigargin, which depletes luminal Ca^{2+} in the ER by the inhibiting Ca^{2+}-ATPase of ER[11].

Thapsigargin (1 μM) caused a rapid increase and a sustained phase of fura-2-fluorescence intensity in the presence of extracellular Ca^{2+} (1.2 mM) (Fig. 1B (a)). However, in the absence of extracellular Ca^{2+}, the sustained phase was abolished (Fig. 1B (a)).

In the absence of extracellular Ca^{2+}, the difference between the ratio at 600 sec and that at the beginning of the determination was significantly lower in thapsigargin-treated cells, than in control cells ($P<0.001$) (Fig. 1B (b)).

The results indicated that the first rapid increase phase was due to Ca^{2+} release from the ER and that the sustained phase was due to Ca^{2+} entry from the extracellular space.

2. *Effects of 2-APB and Ni^{2+} on PGF2α- and thapsigargin-induced $[Ca^{2+}]i$ mobilization in 3T3-L1 preadipocytes*

As shown in Figure 2A (a) and B (a), 2-APB (100 μM) and Ni^{2+} (1 mM) significantly reduced the second phase induced by PGF2α. These inhibitory effects of 2-APB and Ni^{2+} were significantly lower than the

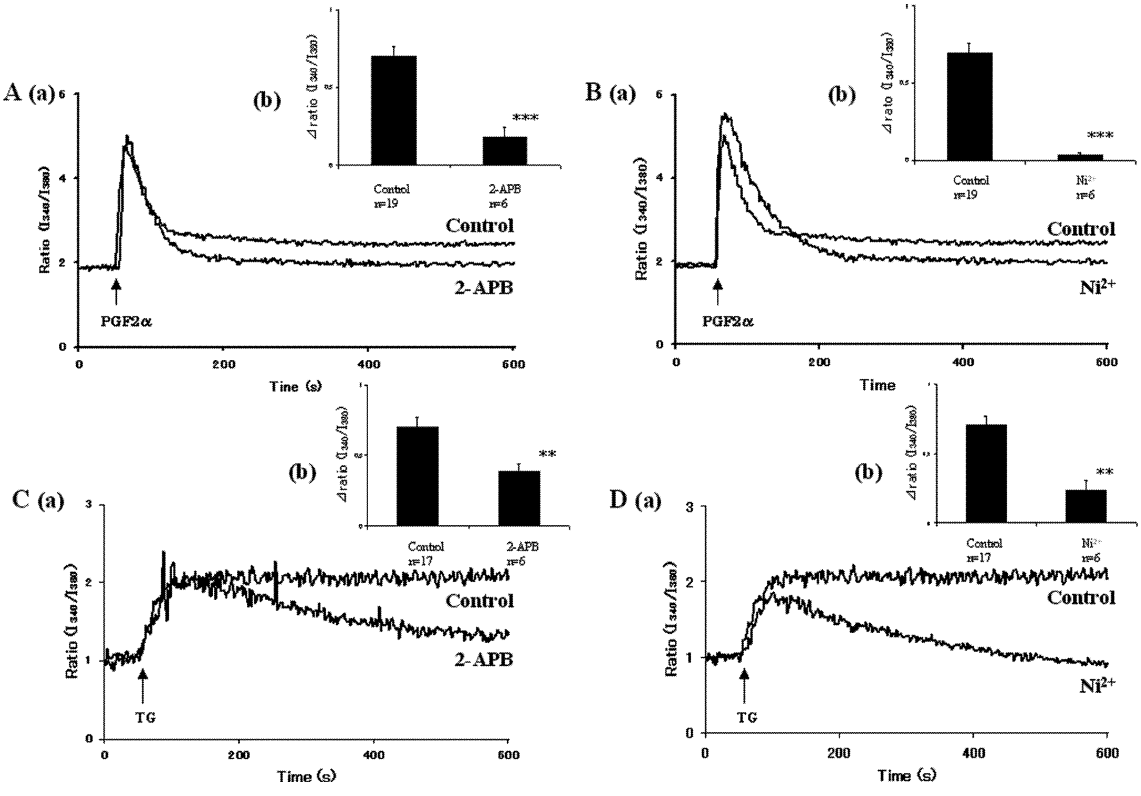

Fig. 2. Effects of 2-APB and Ni^{2+} on PGF2α- and thapsigargin-induced $[Ca^{2+}]i$ mobilization in 3T3-L1 preadipocytes. Cells were pretreated with 2-APB (100 μM) or Ni^{2+} (1 mM) before the addition of PGF2α (1 μM) and thapsigargin (2 μM). A (a): Effect of 2-APB on PGF2α-induced $[Ca^{2+}]i$ mobilization in 3T3-L1 preadipocytes. Typical tracings are shown. A (b): The difference between the ratios of I340/I380 at 600 seconds and at the beginning of fluorescence determination. B (a): Effect of Ni^{2+} on PGF2α-induced $[Ca^{2+}]i$ mobilization in 3T3-L1 preadipocytes. Typical tracings are shown. B (b): The difference between the ratios of I340/I380 at 600 seconds and at the beginning of fluorescence determination. C (a): Effect of 2-APB on thapsigargin-induced $[Ca^{2+}]i$ mobilization in 3T3-L1 preadipocytes. Typical tracings are shown. C (b): The difference between the ratios of I340/I380 at 600 seconds and at the beginning of the fluorescence determination. D (a): Effect of Ni^{2+} on thapsigargin-induced $[Ca^{2+}]i$ mobilization in 3T3-L1 preadipocytes. Typical tracings are shown. D (b): The difference between the ratios of I340/I380 at 600 seconds and at the beginning of fluorescence determination. Each value represents the mean±S.E.; ***, $P<0.001$. **, $P<0.01$.

control value ($P<0.001$) (Fig. 2A (b) and B (b)). 2-APB and Ni^{2+} also attenuated the thapsigargin-induced sustained phase (Fig. 2C (a) and D (a)). Their inhibitory effects were significantly lower than the control value ($P<0.01$) (Fig. 2C (b) and D (b)). These results confirm that SOCE is involved in PGF2α- and thapsigargin-induced Ca^{2+} entry.

3. Effects of cytochalasin D and calyculin A on thapsigargin-induced $[Ca^{2+}]i$ mobilization in 3T3-L1 preadipocytes

Pretreatment with 10 μM cytochalasin D15 for 1 hour did not alter thapsigargin-induced $[Ca^{2+}]$i mobilization (Fig. 3A (a)). Moreover, under the present experimental conditions, pretreatment with calyculin A (100 nM, 30 minutes) did not alter the plateau phase evoked by thapsigargin in 3T3-L1 preadipocytes (Fig. 3B (a)). The difference between the ratios of I340/I380 at 600 seconds and at the beginning of fluorescence determination in cytochalasin D- and calyculin A-treated cells was not significantly lower than in the control (Fig. 3A (b) and B (b)).

4. Effects of cytochalasin D and calyculin A on the actin network in 3T3-L1 preadipocytes

As shown in figure 4, F-actin was visible under a confocal microscope after rhodamine-phalloidin staining. Rhodamine-phalloidin staining showed F-actin fibers distributed parallel or perpendicular to each other in the control cells (Fig. 4A). In the cells treated with cytochalasin D (10 μM, 1 hour), no stress fibers were seen, and depolymerized actin converted into bundles (Fig. 4B). After treatment with calyculin A (100 nM, 30 minutes), F-actin redistributed to the cell periphery to create the dense phase (Fig. 4C).

5. Expression of TRPC mRNA by RT-PCR in 3T3-L1 preadipocytes

Expression of TRPC1 and TRPC2 mRNA was detected after 50 PCR cycles in 3T3-L1 preadipocytes

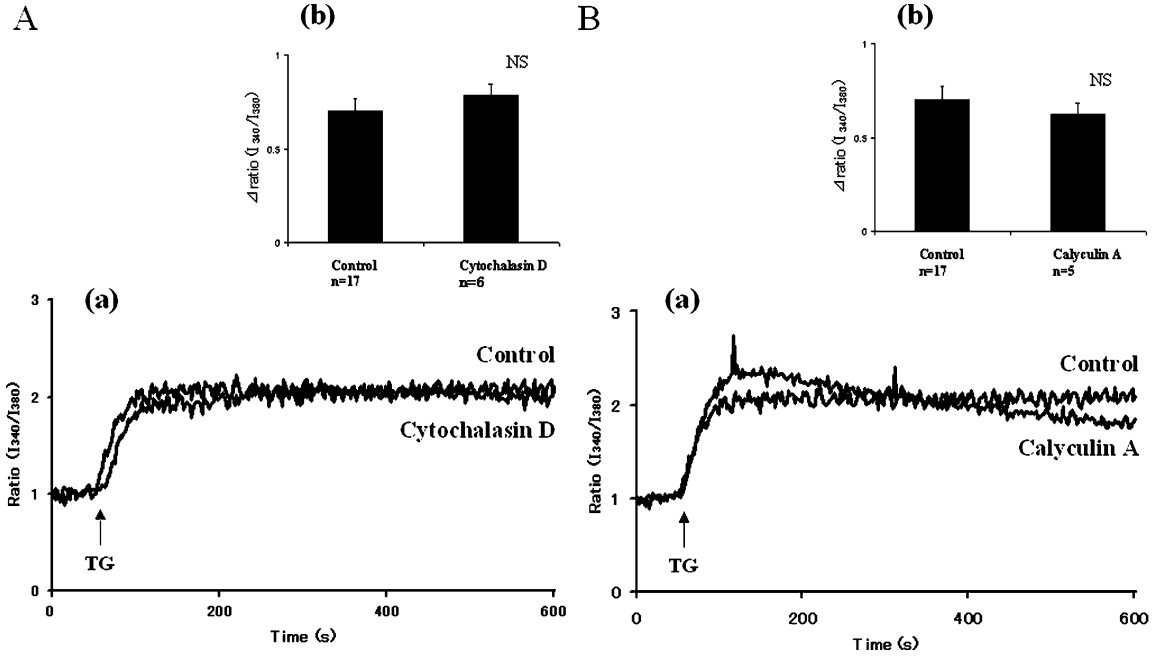

Fig. 3. Effects of cytochalasin D and calyculin A on thapsigargin-induced $[Ca^{2+}]$i mobilization in 3T3-L1 preadipocytes. Cells were pretreated with cytochalasin D (10 μM) for 1 hour and with calyculin A (100 nM) for 30 minutes before the addition of thapsigargin (2 μM). A (a) : Effect of cytochalasin D on thapsigargin-induced $[Ca^{2+}]$i mobilization in 3T3-L1 preadipocytes. Typical tracings are shown. A (b) : The difference between the ratios of I340/I380 at 600 seconds and at the beginning of fluorescence determination. B (a) : Effect of calyculin A on thapsigargin-induced $[Ca^{2+}]$i mobilization in 3T3-L1 preadipocytes. Typical tracings are shown. B (b) : The difference between the ratios of I340/I380 at 600 seconds and at the beginning of fluorescence determination. Each value represents the mean±S.E. ; NS, not significantly different.

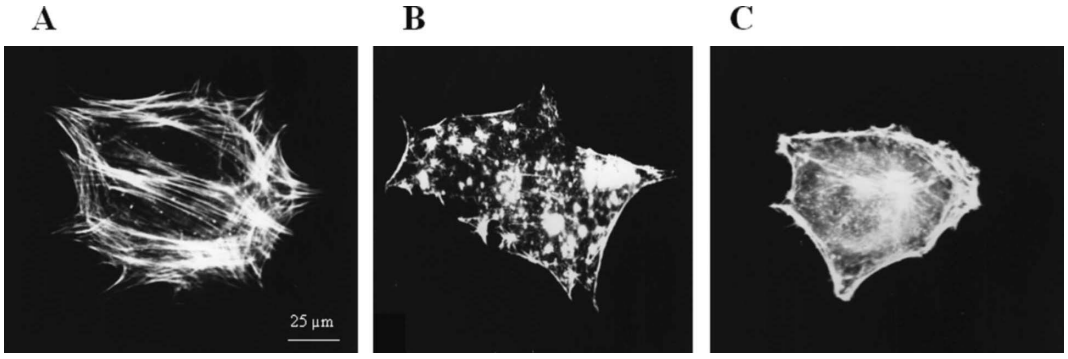

Fig. 4. Effects of cytochalasin D and calyculin A on the actin network in 3T3-L1 preadipocytes.
(A) Control ; (B) Treated with 10 μM cytochalasin D for 1 hour ; (C) Treated with 100 nM calyculin A for 30 minutes

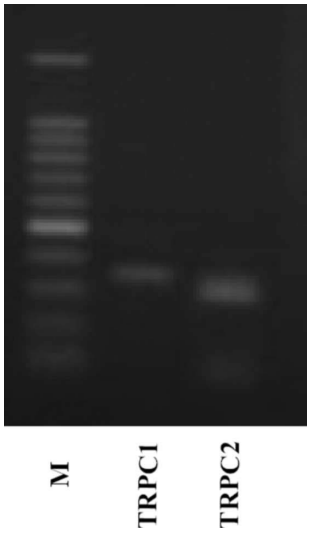

Fig. 5. Expression of TRPC mRNA by RT-PCR in 3T3-L1 preadipocytes. RT-PCR of cDNA was performed using the specific primers shown under Materials and Methods. M : markers.

(Fig. 5). No other TRPC mRNAs were detected (data not shown).

6. PGF2α- and thapsigargin-induced fura-2 fluorescence intensity in the presence of extracellular Sr^{2+} in 3T3-L1 preadipocytes

Under these conditions, the plateau phases induced by PGF2α and thapsigargin were attenuated (Fig. 6A (a) and B (a)). In the presence of extracellular Sr^{2+}, the differences between the ratios of I340/I380 at 600 seconds and at the beginning of fluorescence determination were significantly lower than the control value ($P<0.001$) (Fig. 6A (b) and B (b)).

7. PGF2α- and thapsigargin-induced $[Ca^{2+}]i$ mobilization in 3T3-L1 preadipocytes and 3T3-L1 adipocytes

The PGF2α- and thapsigargin-induced sustained phases in 3T3-L1 adipocytes were similar to those induced in 3T3-L1 preadipocytes (Fig. 7A (a) and B (a)). The differences between the ratios of I340/I380 at 600 seconds and at the beginning of the fluorescence determination did not differ significantly between adipocytes and preadipocytes (Fig. 7A (b) and B (b)).

DISCUSSION

1. SOCE in 3T3-L1 preadipocytes

Ca^{2+} is one of the most important messengers of various cellular functions, such as proliferation, differentiation, gene transcription, and apoptosis[1]. Previous studies have shown that in 3T3-L1 preadipocytes, the increase in $[Ca^{2+}]i$ affects adipocyte differentiation[21]. However, the details of $[Ca^{2+}]i$ mobilization in 3T3-L1 cells are unclear. At least 3 types of Ca^{2+} channel regulate Ca^{2+} entry across the plasma membrane : the voltage-operated Ca^{2+} channel, the receptor-operated Ca^{2+} channel, and the SOCE channel[4]. In this study, a high K^+ concentration failed to trigger Ca^{2+} influx into 3T3-L1 preadipocytes (data not shown). However, Ca^{2+} store depletion from the ER by PGF2α- and thapsigargin induced Ca^{2+} influx. In addition, SOCE inhibitors 2-APB and Ni^{2+} abolished PGF2α- and thapsigargin-induced Ca^{2+} entry. These results indicated that 3T3-L1 preadipocytes possess SOCE but not voltage-

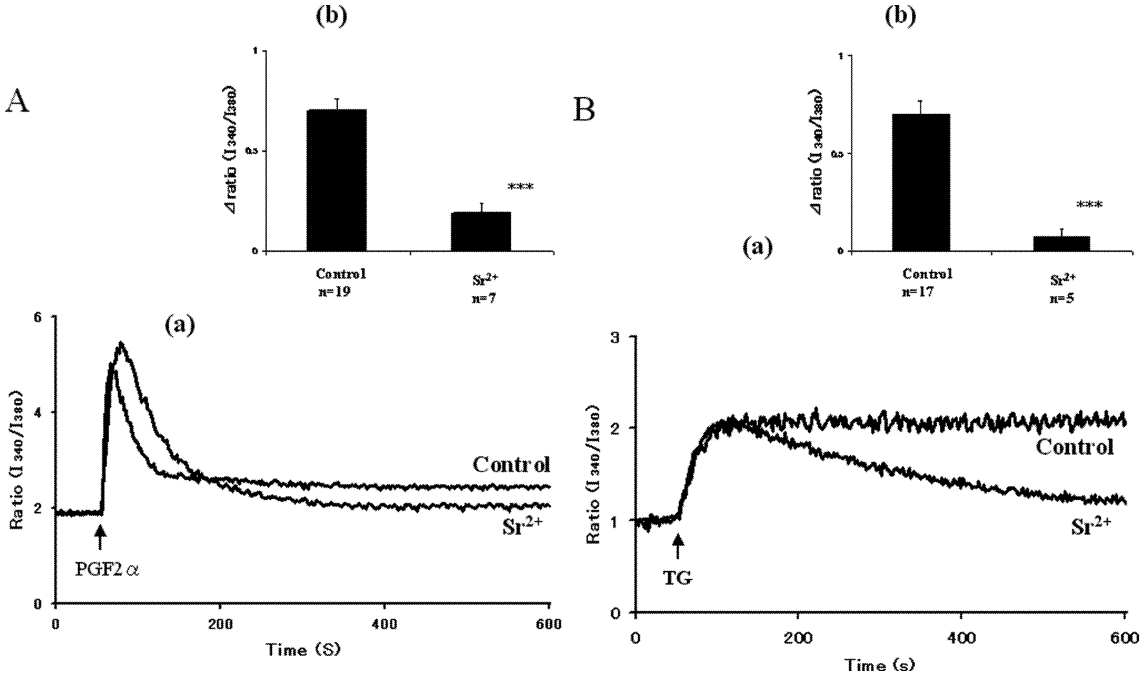

Fig. 6. PGF2α- and thapsigargin-induced fura-2 fluorescence intensity in the presence of extracellular Sr^{2+} in 3T3-L1 preadipocytes. The 1.2 mM Ca^{2+} in the incubation buffer (control) was replaced with 1.2 mM Sr^{2+} (Sr^{2+}). A (a): Effect of PGF2α-induced fura-2-fluorescence intensity in the presence of extracellular Sr^{2+} in 3T3-L1 preadipocytes. Typical tracings are shown. A (b): The difference between the ratios of I340/I380 at 600 seconds and at the beginning of fluorescence determination. B (a): Effect of thapsigargin-induced fura-2-fluorescence intensity in the presence of extracellular Sr^{2+} in 3T3-L1 preadipocytes. Typical tracings are shown. B (b): The difference between the ratios of I340/I380 at 600 seconds and at the beginning of fluorescence determination. Each value represents the mean±S.E.; ***, $P<0.001$.

operated Ca^{2+} channels.

Three mechanisms of SOCE have been hypothesized: a conformational coupling model, a vesicular fusion model, and a diffusible messenger model. In the conformational coupling model, the ER moves to the plasma membrane after luminal Ca^{2+} store depletion by IP3 via the IP3 receptor in the ER[22-25]. The vesicular fusion model suggests that upon Ca^{2+} store depletion, vesicles containing SOCE channels fuse with the plasma membrane, and SOCE is activated[18]. In the diffusible messenger model, SOCE is activated by the diffusible messenger that is produced upon Ca^{2+} store depletion[26]. Among these models, the conformational coupling model and the vesicular fusion model might be closely related to the dynamics of the actin cytoskeleton.

We examined whether the actin cytoskeleton was involved in SOCE in 3T3-L1 preadipocytes. Phase-contrast microscopy revealed that the actin filaments were distributed primarily throughout the cytoplasm in the control cells. After 1 hour of treatment with cytochalasin D, actin filaments became depolymerized. Pretreatment with calyculin A caused reconstruction of the actin filaments in the cell periphery. The conformational coupling model proposes that Ca^{2+} store depletion causes the ER to move towards the plasma membrane to facilitate coupling between the IP3 receptor and the SOCE channel. The actin cytoskeleton is related to SOCE between the ER and the plasma membrane. In spite of the gross structural changes in the actin cytoskeleton after treatment with cytochalasin D and calyculin A, neither agent had any effect on thapsigargin-induced Ca^{2+} mobilization in 3T3-L1 preadipocytes. Therefore, neither the conformational coupling model nor the vesicular fusion model seems to be involved in SOCE in 3T3-L1 preadipocytes. On the other hand, the diffusible messenger model is more likely to occur in these cells. However, the conformational coupling model has been proposed based on research using

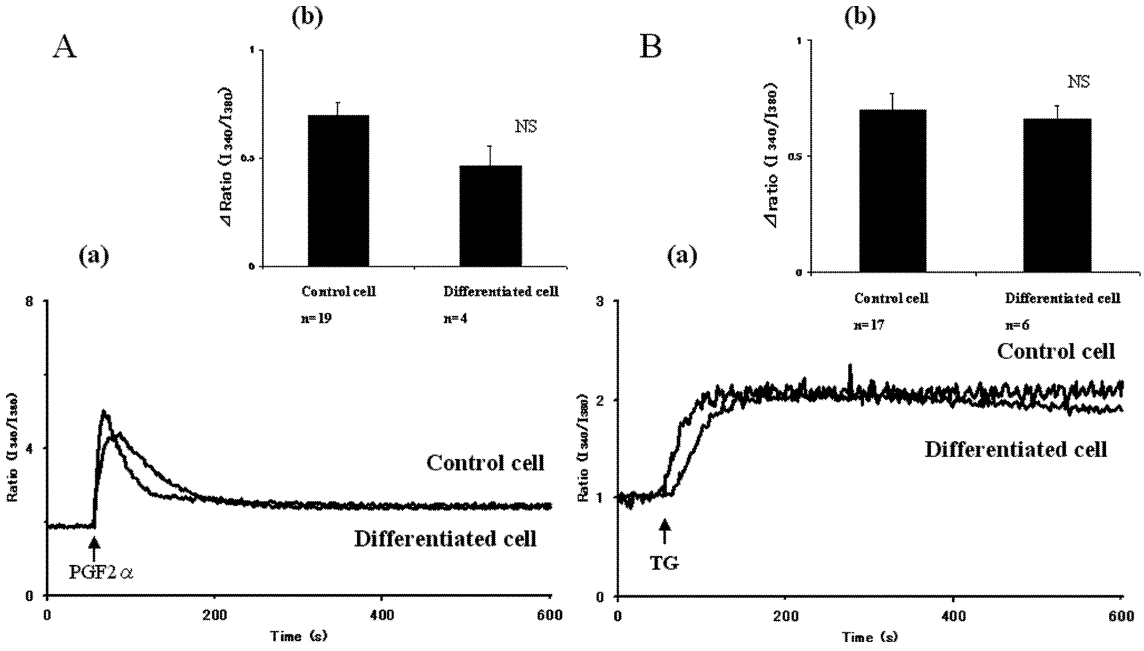

Fig. 7. PGF2α- and thapsigargin-induced $[Ca^{2+}]$i mobilization in 3T3-L1 cells. A (a): Effect of PGF2α on $[Ca^{2+}]$i mobilization in 3T3-L1 preadipocytes (control) and 3T3-L1 adipocytes (differentiated cells). Typical tracings are shown. A (b): The difference between the ratios of I340/I380 at 600 seconds and at the beginning of fluorescence determination. B (a): Effect of thapsigargin on $[Ca^{2+}]$i mobilization in 3T3-L1 preadipocytes (control) and 3T3-L1 adipocytes (differentiated cells). Typical tracings are shown. B (b): The difference between the ratios of I340/I380 at 600 seconds and at the beginning of fluorescence determination. Each value represents the mean±S.E.; NS, not significantly different.

several cell types, such as bovine adrenocortical cells and human platelets[23,24]. This discrepancy might be caused by differences in cell types or cell lines. Therefore, different mechanisms should be considered whenever a SOCE channel is identified.

2. SOCE channels in 3T3-L1 cells

Some studies have indicated that Drosophila Trp and its mammalian homologues, TRPs, form subunits of Ca^{2+}-permeable cation channels that mediate Ca^{2+} influx in response to activation of phospholipase C and internal Ca^{2+} store depletion by agonists[19,20]. The TRP family can be divided into at least 6 subgroups: TRPCs, TRPVs, TRPMs, TRPA1, TRPPs, and TRPMLs[27–30]. Among these channels, TRPCs have previously been proposed to be regulated by the filling state of intracellular Ca^{2+} stores and are the elusive molecular candidates for SOCE[31–33]. We detected TRPC mRNAs expressed in 3T3-L1 preadipocytes and found that mRNA for TRPC1 and TRPC2 were also expressed. Our results reveal the possible involvement of TRPCs in SOCE on 3T3-L1 preadipocytes. While SOCE is high Ca^{2+}-selective[4], TRPCs are not Ca^{2+}-selective, and other divalent ions, such as Sr^{2+}, can pass through. In the presence of extracellular Sr^{2+} in place of extracellular Ca^{2+}, thapsigargin-induced fura-2-fluorescence intensity in the plateau phase was reduced in 3T3-L1 preadipocytes. This finding indicates that TRPCs might not be involved in SOCE in 3T3-L1 preadipocytes.

Recently, stromal interacting molecule (STIM) 1 was identified as an adhesion molecule present in bone marrow stromal cells, and it was hypothesized that this protein might regulate cell formation[34]. STIM1 has an EF-hand Ca^{2+}-binding domain in the luminal site of the ER[35,36]. Recent studies with short interfering (si)RNA have shown that STIM1 is involved in SOCE activity and functions as a Ca^{2+} sensor[37,38]. On the other hand, Fesk et al. have designated Orai1/CRACM1 as a SOCE regulator. They identified Orai1 by using a genome-wide RNA interference screen in Drosophila and a modified linkage of gene

mutation analysis in families with severe combined immune deficiency[39]. Vig et al. have identified CRACM1 by means of RNA interference in Drosophila[40]. The latest reports have demonstrated that STIM1 and Orai1/CRACM1 are essential components of SOCE[41-43]. In a preliminary study, we detected the mRNA of STIM1 in 3T3-L1 preadipocytes (data not shown). Recently, Graham et al. reported the participation of STIM1 in the regulation of preadipocyte differentiation[44]. We hope to determine whether expression of STIM1 and Orai1/CRACM1 is involved in the differentiation of 3T3-L1 preadipocytes.

Although we have shown that SOCE is the major $[Ca^{2+}]i$ mobilization system in 3T3-L1 preadipocytes, whether $[Ca^{2+}]i$ mobilization by SOCE is related to adipocyte differentiation remains unclear. Shi et al. have demonstrated the relationship between $[Ca^{2+}]i$ mobilization and differentiation in human adipose tissue[21]. They have reported that the increase in $[Ca^{2+}]i$ during the early stage of differentiation inhibits adipogenesis while the increase in $[Ca^{2+}]i$ during the later stage of differentiation activates adipogenesis. Under our experimental conditions, thapsigargin-induced Ca^{2+} influx did not differ between 3T3-L1 adipocytes and preadipocytes. The SOCE mechanism itself might be an unchangeable system in 3T3-L1 cells during adipocyte differentiation.

PGFα activates phospholipase C through the FP receptor resulting in IP3-stimulated Ca^{2+}-release from the ER[9,10]. PGF2α-induced SOCE activity in 3T3-L1 preadipocytes does not differ from that in differentiated cells. PGF2α production has been reported to decrease during adipocyte differentiation[45,46]. The levels of FP receptor do not change during adipocyte differentiation in 3T3-L1 cells[45]. In addition, PGF2α receptor regulatory protein (FPRP) has been identified as a transmembrane protein that inhibits binding of the FP receptor to its ligand[47]. FPRP is not present in preadipocytes but it is induced during adipocyte differentiation in 3T3-L1 cells[48]. Our results and those of previous reports suggest that SOCE activation through PGF2α might be involved in adipogenesis in 3T3-L1 cells and that the regulation of endogenous PGF2α production and FPRP levels, not FP receptor levels, play important roles in the adipocyte differentiation of 3T3-L1 cells into adipocytes. Further studies are needed to clarify this possibility in 3T3-L1 cells.

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