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Role of Ca^{2+}-independent phospholipase A_2 and cytochrome P-450 in store-operated calcium entry in 3T6 fibroblasts

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Abstract

Store-operated calcium (SOC) channels and capacitative Ca^{2+} entry play a key role in cellular functions, but their mechanism of activation remains unclear. Here, we show that thapsigargin induces [^{3}H] arachidonic acid (AA) release, $^{45}Ca^{2+}$ influx and a subsequent enhancement of intracellular calcium concentration ($[Ca^{2+}]_i$. Thapsigargin-induced elevation of $[Ca^{2+}]_i$ was inhibited by cytochrome P-450 inhibitors and by cytochrome P-450 epoxygenase inhibitor and was reverted by 11,12 EET addition. However, cyclooxygenase and lipoxygenase inhibitors have no effect. Moreover, we observed that four EETs were able to induce $^{45}Ca^{2+}$ influx. Finally, we reported that the effect of 11,12 EET on $^{45}Ca^{2+}$ influx was sensible to receptor-operated Ca^{2+} channel blockers ($NiCl_2$, $LaCl_3$) but not to voltage-dependent Ca^{2+} channel blocker as verapamil. Thus, AA released by Ca^{2+}-independent phospholipase A_2 and AA metabolism through cytochrome P-450 pathway may be crucial molecular determinant in thapsigargin activation of SOC channels and store-operated Ca^{2+} entry pathway in 3T6 fibroblasts. Moreover, EETs, the main cytochrome P-450 epoxygenase metabolites of AA, are involved in thapsigargin-stimulated Ca^{2+} influx. In summary, our results suggest that EETs are components of calcium influx factor(s).

Keywords: Calcium channels; Capacitative calcium entry; Epoxyeicosatrienoic acids; Arachidonic acid; Eicosanoids

1. Introduction

In many cells, calcium (Ca^{2+}) signals comprise both release of Ca^{2+} from intracellular pools and its entry across the plasma membrane. Both events are closely coupled as the entry of Ca^{2+} is triggered by the emptying of Ca^{2+} from pools in response to inositol trisphosphate (InsP3) [1]. In intraluminal Ca^{2+} levels largely vary in response to InsP3-induced Ca^{2+} release from endoplasmic reticulum (ER) [2]. The decrease in Ca^{2+} stored within the ER appears to be the primary determinant for activating the opening of store-operated Ca^{2+} (SOC) channels in the plasma membrane, thus allowing the entry of Ca^{2+}, which enhances cytosolic Ca^{2+} signals and the loading of intracellular pools [1]. This process is called ''capacitative calcium entry'' (CCE) or ''store-operated calcium entry'' [3] and the signal for SOC channel activation appears to be the depletion of ER Ca^{2+} stores.

Although distinct conductances may be responsible for this store-operated or capacitative pathway in several cell types, the most thoroughly characterized are the so-called Ca^{2+} release-activated Ca^{2+} (CRAC) channels. There are very low-conductance, highly Ca^{2+}-selective, channels whose gating is independent of voltage but entirely dependent on the depletion of internal Ca^{2+} stores [4]. Such CRAC channels have been described in a wide variety of non-excitable cells [4]. Their molecular identity and precise mechanism of activation, however, remain unclear. A non-selective SOC channel of small, but resolvable 3 pS conductance has also been described in vascular smooth muscle cells and platelets [5].

The ER accumulates Ca^{2+} via the function of intracellular sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase Ca^{2+} pump proteins [6]. These ATPase pumps are extremely sensitive to the Ca^{2+} pump blocker, thapsigargin [7], which binds to intracellular Ca^{2+} pumps with high affinity,

Abbreviations: AA, arachidonic acid; $AACOCF_3$, arachidonyl trifluoromethylketone; ARC, arachidonate-regulated Ca^{2+}; BEL, bromoenol lactone; CCE, capacitative calcium entry; CRAC, calcium release-activated Ca^{2+} channels; CIF, calcium influx factor; EET, epoxyeicosatrienoic acid; ER, endoplasmic reticulum; FCS, fetal calf serum; $InsP_3$, inositol trisphosphates; $[Ca^{2+}]_i$, intracellular calcium concentrations; $iPLA_2$, calcium-independent phospholipase A_2; PLA_2, phospholipase A_2; PPOH, 6-(2 propargyloxyphenyl) hexanoic acid; SOC, store-operated calcium

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resulting in a virtually irreversible inhibition of Ca^{2+} accumulation within the ER [8]. Thus, CCE can be alternatively induced by emptying the Ca^{2+} store with the use of inhibitors of ER Ca^{2+} ATPase like thapsigargin [4]. However, the biochemical nature of the coupling between calcium content of the ER and the activity of SOCs remain unclear.

Three main hypotheses have been proposed to explain signalling between the ER and the plasma membrane. First, direct conformational interaction between the ER membranes and SOCs may be triggered by the activation of InsP3-sensitive receptors in the ER [1]. However, this model was challenged by the findings that cells lacking the InsP3 receptor have normal SOC activity [9]. Second, a secretion-like coupling model based on the fact that the reorganization of the cortical actin cytoskeleton modulates CCE was put forward [10]. However, Bakowski et al. [11] showed that none of the manoeuvres that alter the actin cytoskeleton affects Icrac, the best characterized SOCs. The third hypothesis suggests the existence of a soluble messenger released into the cytosol after release of calcium from the ER, termed calcium influx factor (CIF). CIF induces influx of extracellular calcium through second messenger operated channels, thus coupling calcium entry to the depletion of internal stores. To date, the chemical nature of CIF has not been resolved. However, cytochrome P-450 metabolites have been proposed to act as CIFs [12], since cytochrome P-450 inhibitors inhibit CCE. In this way, several recent studies provide more evidence for the role of arachidonic acid metabolites released by cytochrome P-450 epoxygenase pathway [13]. In the current study, we observed that arachidonic acid (AA) released by Ca^{2+}-independent phospholipase A_2 is involved in thapsigargin activation of store-operated Ca^{2+} entry pathway. Furthermore, we present additional evidence that AA metabolites of epoxygenase pathway like 11,12 epoxyeicosatrienoid acid (11,12 EET) behave as CIF in 3T6 fibroblasts.

2. Materials and methods

2.1. Materials

[5,6,8,9,11,12,14,15-^{3}H]AA (200–240 Ci/mmol) and ^{45}calcium chloride (370 GBq/g) were from American Radiolabeled Chemicals Inc. Arachidonic acid, thapsigargin, ketoprofen, baicalein, proadifen (SKF-525A), 6-(2-propargyloxyphenyl) hexanoic acid (PPOH), 11,12-epoxy (5*Z*,8*Z*,14*Z*)-eicosatrienoic acid (11,12 EET), 17-octadecynoic acid (17-ODYA), verapamil, $NiCl_2$, $LaCl_3$, fura-II/AM and pluronic F-127 were purchased from Sigma Chemical Co. Bromoenol lactone (BEL) and arachidonyl trifluoromethylketone ($AACOCF_3$) were acquired from Alexis Corp. Valeryl salicylate, NS-398, 5,6 EET, 8,9 EET and 14,15 EET were from Cayman Chemical Co. RPMI 1640 medium, fetal calf serum (FCS), penicillin G, streptomycin and trypsin/EDTA were supplied by Bio Whittaker, Europe. All other reagents were of analytical grade.

2.2. Cell culture

Murine 3T6 fibroblasts (American Type Culture Collection, CL96) (passages 61–66) were grown as described elsewhere [14], in RPMI 1640 containing 10% FCS, penicillin (100 U/mL) and streptomycin (100 μg/mL), in a humidified atmosphere of 95% air–5% CO_2 at 37 °C. Cells were harvested with trypsin/EDTA and passed to tissue culture 100 mm or 60 mm-dishes for experimental purposes.

2.3. Incorporation and release of [^{3}H]AA

After cell replication and FCS starvation (6 h), the medium was removed and replaced by 0.5 mL RPMI containing 0.1% fatty acid-free BSA and 0.1 μCi [^{3}H]AA for 24 h. [^{3}H]AA incorporation was performed in preconfluent cultures (3000 cells/cm^2). Cells were then washed three times with medium containing 0.5% BSA to remove unincorporated [^{3}H]AA. After a study period, the medium was removed for analysis of the radioactivity released. The amount of [^{3}H]AA released into the medium was expressed as a percentage of cell-incorporated [^{3}H]AA, which was determined in solubilized cells.

2.4. Measurement of $^{45}Ca^{2+}$ influx

To measure the influx of calcium from extracellular medium, the medium was removed from cell culture and replaced by 0.5 mL RPMI containing 0.5% fatty acid-free BSA and $^{45}CaCl_2$ (0.25 μCi/mL). Cells were incubated for 15 min at 37 °C and cells were washed three times with medium containing 0.5% BSA. To determine the cellular amount of $^{45}Ca^{2+}$, washed cells were solubilized with Triton X-100 (1%) and $^{45}Ca^{2+}$ activity was analyzed by liquid scintillation counting.

2.5. Measurement of intracellular calcium concentrations

Cells grown on glass slices were washed in Ca^{2+}-free Tyrode's buffer containing 0.35% albumin. Thereafter cells were incubated with Fura-II/AM (3 μM) and pluronic F-127 (0.02%, v/v) in RPMI at 37 °C for 45 min in the dark. They were washed with Ca^{2+}-free Tyrode's buffer and cultured in this buffer with 1 mM $CaCl_2$. Fluorescence was monitored on a spectrofluorometer (Perkin-Elmer 650-40) with dual excitation wavelengths of 340 and 380 nm and an emission wavelength of 520 nm. Intracellular calcium concentrations ($[Ca^{2+}]_i$) were calculated as described by Grynkiewicz et al. [15].

2.6. Statistical analysis

Results are expressed as mean ± S.E.M. Differences between non-treated and treated cells were analyzed by the Student's *t*-test followed by the least significant difference test as appropriate.

3. Results

3.1. Effect of thapsigargin on $[Ca^{2+}]_i$ and $^{45}Ca^{2+}$ influx in 3T6 fibroblast cultures

Thapsigargin is a pharmacological agent that inhibits the Ca^{2+}-ATPase of the intracellular calcium store and activates capacitative calcium influx independently of phospholipase C [7]. Hundred nanomolars of thapsigargin rapidly increased 3T6 fibroblast $[Ca^{2+}]_i$, which remained above baseline for at least 15 min (Fig. 1A). The extent of the enhancement of $[Ca^{2+}]_i$ by thapsigargin depended on the presence of extracellular Ca^{2+}. Thus, the thapsigargin-induced increase in $[Ca^{2+}]_i$ decreased when Ca^{2+} was removed from extracellular medium by EGTA addition. Therefore, the enhancement of $[Ca^{2+}]_i$ by thapsigargin may be due, at least in part, to the higher influx of extracellular Ca^{2+}. To test this hypothesis, we determined the effect of thapsigargin on uptake of $^{45}Ca^{2+}$ into 3T6 fibroblasts. Thapsigargin significantly raised $^{45}Ca^{2+}$ influx, which reached a plateau within 5 min (Fig. 1B).

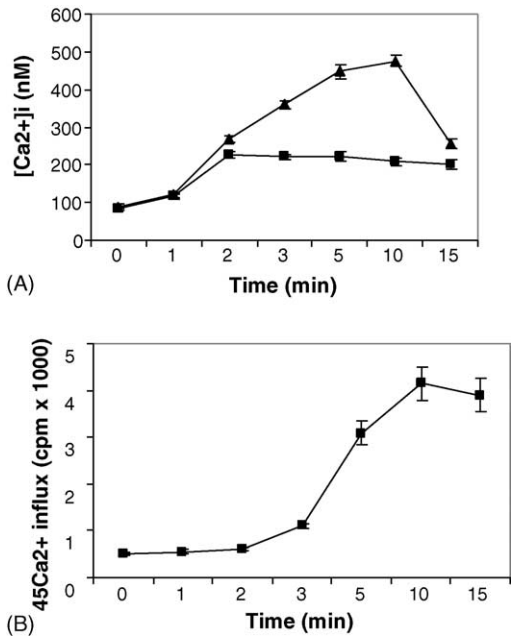

Fig. 1. $[Ca^{2+}]_i$ and influx of $^{45}Ca^{2+}$ to 3T6 fibroblasts treated with thapsigargin. Cells were stimulated with thapsigargin (100 nM) for 15 min at 37 °C in presence (triangle) or absence (square) of extracellular calcium ($CaCl_2$ 1 mM) (2 mM EGTA was added to quelate calcium). Then, $[Ca^{2+}]_i$ (A) or $^{45}Ca^{2+}$ influx (B) was measured. Results represent the mean ± S.E.M. from three to four determinations performed in duplicate.

3.2. Effect of thapsigargin on [^{3}H]AA release

If a metabolite of AA is a component of CIF, then AA should be released in response to depletion of intracellular calcium stores. Using [^{3}H]AA-labeled fibroblasts, we investigated AA release in response to thapsigargin. We observed that thapsigargin triggered [^{3}H]AA release, which reached a plateau at 5 min. Interestingly, the removal of extracellular Ca^{2+} did not significantly alter the [^{3}H]AA release induced by thapsigargin (Fig. 2).

[^{3}H]AA release induced by thapsigargin was inhibited by $AACOCF_3$, an non-selective and reversible inhibitor of high-molecular weight phospholipase A_2 (PLA_2) [16] and by BEL, a suicidal substrate for calcium-independent phospholipase A_2 with a specificity for $iPLA_2$ about 1000 times higher than for other PLA_2 isoforms [17] (Fig. 3A). These results point to the involvement of $iPLA_2$ in thapsigargin induced-[^{3}H]AA release and suggest that $iPLA_2$ activity is associated with the release of calcium from intracellular stores.

To determine the role of AA cascade on $^{45}Ca^{2+}$ uptake induced by thapsigargin, we determined $^{45}Ca^{2+}$ influx in presence of the above treatments that modulate AA mobilization. The effect of BEL and $AACOCF_3$ on capacitative Ca^{2+} influx was concentration-dependent. BEL and $AACOCF_3$ treatments significantly inhibited thapsigargin-induced Ca^{2+} influx (Fig. 3B). These results suggest that the activation of CCE in 3T6 fibroblast depends on the AA release induced by $iPLA_2$.

3.3. Thapsigargin-induced elevation of $[Ca^{2+}]_i$ is blocked by cytochrome P-450 inhibitors

The thapsigargin-stimulated increase in $[Ca^{2+}]_i$ depends on the release from intracellular stores followed by capacitative influx of extracellular calcium. If capacitative calcium influx is mediated by AA metabolites, thapsigargin-induced $^{45}Ca^{2+}$ influx should be inhibited by blockade of AA metabolism. To test this hypothesis we used ketoprofen as cyclooxygenase inhibitor [18], valeryl salicylate as cyclooxygenase-1 inhibitor [19], NS-398 as cycloox-

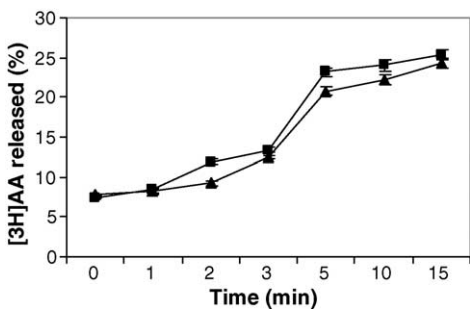

Fig. 2. Effect of thapsigargin on [^{3}H]AA release in 3T6 fibroblast cultures. Cells were stimulated with thapsigargin (100 nM) for 15 min at 37 °C in presence (square) or absence (triangle) of extracellular calcium ($CaCl_2$ 1 mM) (2 mM EGTA was added to quelate calcium). Results represent the mean ± S.E.M. from three to four determinations performed in duplicate.

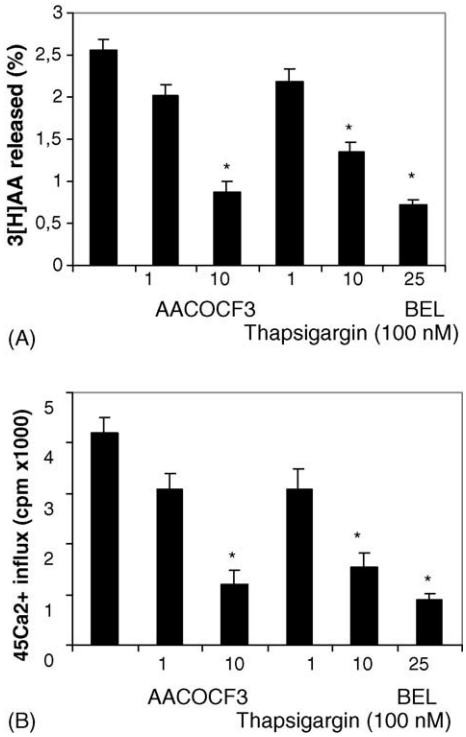

Fig. 3. Effect of $AACOCF_3$ and BEL on [^{3}H]AA release and $^{45}Ca^{2+}$ influx induced by thapsigargin. 3T6 fibroblast was pre-incubated with $AACOCF_3$ (1–10 μM) or BEL (1–25 μM) for 15 min before the addition of thapsigargin. Cells were then incubated with thapsigargin for 15 min at 37 °C and [^{3}H]AA release (A) or $^{45}Ca^{2+}$ influx (B) were measured. The background of [^{3}H]AA release from untreated cells (9 ± 2% of [^{3}H]AA incorporated) was subtracted from all data. Bars represent the mean ± S.E.M. from three to four determinations performed in duplicate. $^{*}P < 0.05$ respect to non-treated cells.

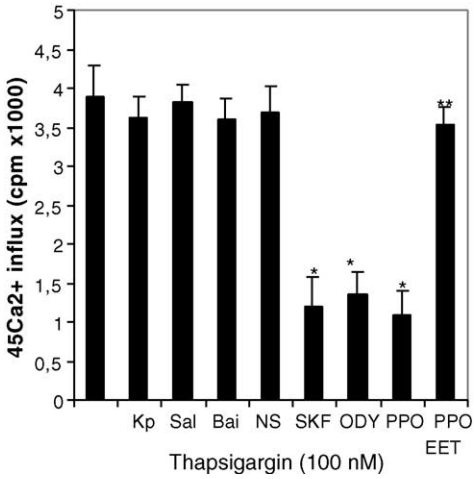

Fig. 4. Effect of AA metabolism inhibitors on $^{45}Ca^{2+}$ influx induced by thapsigargin. 3T6 fibroblasts were preincubated with ketoprofen (Kp, 0.5 μM), valeryl salicylate (Sal, 1 mM), NS-398 (NS, 5 μM), baicalein (Bai, 30 μM), SKF 525A (SKF, 10 μM), PPOH (10 μM) or PPOH (10 μM) plus EET (1 μM) for 60 min and $^{45}Ca^{2+}$ influx was stimulated by thapsigargin (100 nM) for 15 min at 37 °C. Bars represent the mean ± S.E.M. from three to four determinations performed in duplicate. $^{*}P < 0.05$ respect to non-treated cells; $^{**}P < 0.05$ respect to PPOH-treated cells.

ygenase-2 inhibitor [20], baicalein as lipoxygenase inhibitor [21] and SKF 525A and 17-ODYA as cytochrome P-450 inhibitors [22,23]. Neither cyclooxygenase nor lipoxygenase inhibitors affected the $^{45}Ca^{2+}$ influx induced by thapsigargin. However, SKF 525A or 17-ODYA, inhibitors of cytochrome P-450-dependent metabolism of AA [24], significantly inhibited $^{45}Ca^{2+}$ uptake in 3T6 fibroblast cultured with thapsigargin (Fig. 4). In addition to cyclooxygenase and lipoxygenase pathways, cytochrome P-450 also catalyzes the in vivo metabolism of AA to biologically active compounds. Thus, olefin epoxidation produces epoxyeicosatrienoic acids (EETs), allylic oxidation generates hydroxyeicosatetraenoic acids (HETEs) and w-1-hydroxylation yields 20-HETE [25]. PPOH, a selective inhibitor of EETs synthesis [26] also inhibited $^{45}Ca^{2+}$ influx induced by thapsigargin, which was reverted by exogenous addition of 11,12 EET. These data suggest that this AA metabolite is involved in the capacitative calcium entry process. Considering that Rzigalinski et al. [13] reported that 5,6 EET mediates Ca^{2+} influx, we proposed to study the effect of EETs on $^{45}Ca^{2+}$ influx in 3T6 fibroblast cultures. As we can see in Table 1, the four EETs were able to induce $^{45}Ca^{2+}$ influx although 11,12 EET induced the more appreciable effect.

We used verapamil and Ni^{2+} or La^{3+} to investigate the role of voltage-operated Ca^{2+} channel (L-type) and receptor Ca^{2+} channel, respectively, in the thapsigargin- and EET-induced increase in $^{45}Ca^{2+}$ influx in 3T6 fibroblasts. $NiCl_2$ (1–5 mM) or $LaCl_3$ (10 μM), receptor-operated Ca^{2+} channel blockers [27], inhibited the $^{45}Ca^{2+}$ uptake induced by thapsigargin or EET. However, verapamil, a voltage-dependent Ca^{2+} channel blocker [28] did not attenuate the increase in $^{45}Ca^{2+}$ influx induced by both agonists (Table 2), suggesting that Ca^{2+} influx through voltage-dependent Ca^{2+} channels does not mediate the thapsigargin and/or EET-induced increase in 3T6 fibroblast $^{45}Ca^{2+}$ influx.

4. Discussion

The PLA_2 family consists of a growing number of enzymes that catalyze the hydrolysis of the ester bound

Table 1
Effect of EETs on $^{45}Ca^{2+}$ influx

$^{45}Ca^{2+}$ influx (cpm × 1000)	
Control	0.51 ± 0.02
5,6 EET (0.1 μM)	1.18 ± 0.02*
5,6 EET (1 μM)	1.59 ± 0.03*
8,9 EET (0.1 μM)	1.32 ± 0.02*
8,9 EET (1 μM)	1.78 ± 0.03*
11,12 EET (0.1 μM)	2.01 ± 0.04*
11,12 EET (1 μM)	2.38 ± 0.06*
14,15 EET (0.1 μM)	1.87 ± 0.03*
14,15 EET (1 μM)	2.19 ± 0.05*

Cells were incubated in presence of EETs for 15 min and $^{45}Ca^{2+}$ uptake was measured. Data are means ± S.E.M. of three determinations performed in duplicate.

* $P < 0.05$ respect to control cells.

Table 2
Effect of verapamil, $NiCl_2$ and $LaCl_3$ on $^{45}Ca^{2+}$ influx induced by thapsigargin or EET

$^{45}Ca^{2+}$ influx (cpm × 1000)	
Control	0.52 ± 0.03
Thapsigargin	$3.93 \pm 0.12^{*}$
Thapsigargin + verapamil (5 μM)	3.75 ± 0.11
Thapsigargin + verapamil (50 μM)	3.68 ± 0.07
Thapsigargin + $NiCl_2$ (1 mM)	$3.15 \pm 0.13^{**}$
Thapsigargin + $NiCl_2$ (5 mM)	$2.59 \pm 0.11^{**}$
Thapsigargin + $LaCl_3$ (10 μM)	$2.42 \pm 0.13^{**}$
11,12 EET	$2.42 \pm 0.09^{*}$
11,12 EET + verapamil (50 μM)	2.36 ± 0.05
11,12 EET + $NiCl_2$ (5 mM)	$1.46 \pm 0.11^{**}$
11,12 EET + LaQ (10 μM)	$1.52 \pm 0.09^{**}$

Cells were incubated in presence of verapamil, $NiCl_2$ or $LaCl_3$ for 30 min. Cells were then stimulated with thapsigargin (100 nM) or 11,12 EET (1 μM) for 15 min and $^{45}Ca^{2+}$ uptake was measured. Data are means ± S.E.M. of three determinations performed in duplicate.

* $P < 0.05$ respect to control cells.

** $P < 0.05$ respect to non-treated cells stimulated with thapsigargin or EET.

at the sn-2 position of glycerophospholipids and the release of AA. Numerous intracellular and secreted PLA_2 have been described to date. The cytosolic 85-kDa PLA_2 ($cPLA_2$) is regulated by physiological intracellular calcium concentrations and phosphorylation and activated by cytokines and growth factors, whereas $iPLA_2$ has been proposed as a housekeeping enzyme involved in the remodelling of membrane phospholipids [29]. We previously reported the expression of both PLA_2 families in 3T6 fibroblast cultures [30]. Furthermore, we described the expression of both COXs and the prostaglandin synthesis by 3T6 fibroblasts [14]. These cells did not produce appreciable amounts of leukotrienes. However, dermal fibroblasts are able to metabolize AA by cytochrome P-450 pathway [31] and we also observed this event in 3T6 fibroblast cultures (data not shown). $iPLA_2$ has not been regarded as a relevant component of store-operated Ca^{2+} influx pathway in the models proposed so far (see references [3,32] for recent review of the existing models). However, Smani et al. [33] have recently proposed that $iPLA_2$ activity is required for activation of SOC and capacitative Ca^{2+} influx in non-excitable cell lines like smooth muscle cells, platelets and Jurkat T-lymphocytes. In this way, our study demonstrates that AA release by BEL sensitive $iPLA_2$ is a crucial determinant in activation of SOC and capacitative Ca^{2+} influx in 3T6 fibroblasts.

AA, a main product of PLA_2 activity, including $iPLA_2$, does not seem to be involved in direct activation of SOC channels and CCE [34]. It is now well established that AA has its own specific target, the so-called arachidonate-regulated Ca^{2+} (ARC) selective channels [35,36], which may be responsible for a part of agonist-induced Ca^{2+} influx. Biophysical analysis reveals that ARC and CRAC channels represent entirely distinct conductances with several unique characteristics, especially the fact that activation of the ARC channels is entirely independent of store depletion [35] and is regulated by receptor-activated increase in AA. This was detected after the stimulation of the cells with growth factors [37]. These findings have lead to the conclusion that ARC and CRAC channels represent co-existing but independent Ca^{2+} influx pathways. Alternatively, the products of AA metabolism may be involved in the store-operated pathway. It has recently been shown that inhibition of the lipoxygenase pathway reduces CRAC channel current in RBL cells [38], but the exact mechanism of such effect is not clear. Our results suggest that AA metabolites by cytochrome P-450 are involved in these events in 3T6 fibroblasts, but not cyclooxygenase or lipoxygenase pathways.

Randriamanpita and Tsien [39] isolated a moderately hydrophobic CIF-like substance of molecular weight lower than 500 from stimulated Jurkat cells and Parekh et al. [40] reported that CIF formation involves a phosphatase and diffusible second messenger. Cytochrome P-450 activity has also been coupled to the formation of CIF [12]. In addition to cyclooxygenase and lipoxygenase pathways, cytochrome P-450 also catalyses the metabolism of AA to biologically active compounds by three types of NADPH-dependent oxidative reactions: epoxidation produces EETs, allylic oxidation generates 5,8,9,11,12,15 HETEs and hydroxylation yields 20 HETE [25]. Our results show that SKF 525A and 17-ODYA decreased the $^{45}Ca^{2+}$ uptake induced by thapsigargin. This effect was also observed when we used PPOH to inhibit epoxidation activity of cytochrome P-450 and was reverted when we added exogenous 11,12 EET. Cytochrome P450 epoxygenases can metabolizes AA to four regioisomeric EETs: 5,6 EET, 8,9 EET, 11,12 EET and 14,15 EET [41]. Our results shown that four EETs induce $^{45}Ca^{2+}$ influx. These results support the hypothesis that EETs are CIFs in 3T6 fibroblasts as reported in corneal endothelial cells [42] and in smooth muscle cells [43]. Moreover, 11,12 EET-activated $^{45}Ca^{2+}$ influx was sensitive to Ni^{2+} or La^{3+} but insensitive to the organic Ca^{2+} antagonist verapamil, which is characteristic of SOCs [44]. EETs may be stimulated directly or through the production of second messenger SOC channel activity. Or else EET may be rapidly incorporated into cellular phospholipids and the presence of EET is membrane phospholipids may alter fibroblast signalling, including Ca^{2+} transport. Karara et al. [45] proposed a functional role for cytochrome P-450 in the control of cell membrane microenvironment structure and hence its functional properties. Thus, EET-phospholipids modified the physicochemical properties of the membrane lipid bilayer. These events may result in a more fluid state, and changes in the lipid bilayer order, fluidity and volume may regulate the flux of ions (e.g., Ca^{2+}) across the membrane permeability barrier. In this regard, the inhibition of reconstituted L-type calcium channels by synthetic 1-palmitoyl-2-(11,12) epoxieicosatrienoyl phosphatidylcholine has recently been reported [46]. EETs have been favourite candidates for the endothelium-derived hyperpolarizing

factor that activates smooth muscle K^+ channels [47]. Thus, EETs may enhance CCE by hyperpolarizing the cell membrane through the activation of K^+ channels. Interestingly, Xiao et al. [48] recently reported that overexpression of cytochrome P-450 2J2 increases EET levels and L-type Ca^{2+} currents in cardiomyocytes via a mechanism that involves cAMP-protein kinase A-dependent phosphorylation of the L-type Ca^{2+} channel.

In conclusion, the major finding in this study is that AA release-induced by $iPLA_2$ and EETs synthetized from AA by cytochrome P-450 pathway could be involved in thapsigargin-stimulated Ca^{2+} influx in 3T6 fibroblast. Moreover, there is evidence that EETs are components of calcium influx factors.

Acknowledgements

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