Introduction

Lysophosphatidic acid (LPA), a water-soluble bioactive lysophospholipid, is present in both the serum and the cytosol compartment of the cell. The cytosolic LPA is formed as a metabolite of phosphatidic acid (PA) by the action of phospholipase A2 [1], whereas, the serum LPA is mainly formed due to hydrolysis of lysophosphatidylcholine by phospholipase D (PLD) and is released into the serum from platelets and fibroblasts [2]. The concentration of LPA varies from 1 to 5 µM under physiological conditions and can reach up to 20 µM in response to injury and inflammation [3].

Mechanisms of the lysophosphatidic acid-induced increase in [Ca^{2+}]_i in skeletal muscle cells

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Abstract

Although lysophosphatidic acid (LPA) is known to increase intracellular free calcium concentration ([Ca^{2+}]_i) in different cell types, the effect of LPA on the skeletal muscle cells is not known. The present study was therefore undertaken to examine the effect of LPA on [Ca^{2+}]_i in C2C12 cells. LPA induced a concentration and time dependent increase in [Ca^{2+}]_i, which was inhibited by VPC12249, VPC 32183 and dioctanoyl glycerol pyrophosphate, LPA1/3 receptor antagonists. Pertussis toxin, a G protein inhibitor, also inhibited the LPA-induced increase in [Ca^{2+}]_i. Inhibition of tyrosine kinase activities with tyrphostin A9 and genistein also prevented the increase in [Ca^{2+}]_i due to LPA. Likewise, wortmannin and LY 294002, phosphatidylinositol 3-kinase (PI3-K) inhibitors, inhibited [Ca^{2+}]_i response to LPA. The LPA effect was also attenuated by ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA), an extracellular Ca^{2+} chelator, Ni^{2+} and KB-R7943, inhibitors of the Na⁺-Ca^{2+} exchanger; the receptor operated Ca^{2+} channel (ROC) blockers, 2-aminoethoxydiphenyl borate and SK&F 96365. However, the L-type Ca^{2+} channel blockers, verapamil and diltiazem; the store operated Ca^{2+} channel blockers, La^{3+} and Gd^{3+}; a sarcoplasmic reticulum calcium pump inhibitor, thapsigargin; an inositol trisphosphate receptor antagonist, xestospongin and a phospholipase C inhibitor, U73122, did not prevent the increase [Ca^{2+}]_i due to LPA. Our data suggest that the LPA-induced increase in [Ca^{2+}]_i might occur through G protein coupled LPA1/3 receptors that may be linked to tyrosine kinase and PI3-K, and may also involve the Na⁺-Ca^{2+} exchanger as well as the ROC. In addition, LPA stimulated C2C12 cell proliferation via PI3-K. Thus, LPA may be an important phospholipid in the regulation of [Ca^{2+}]_i and growth of skeletal muscle cells.

Keywords: lysophosphatidic acid • skeletal muscle cell • intracellular free calcium • receptor operated Ca^{2+}-channels • sarcolemmal Na⁺-Ca^{2+} exchanger
G-protein coupled receptors (GPCRs) are known to regulate a diverse range of physiological processes in response to a variety of agonists [4], however, the function of the majority of GPCRs expressed in skeletal muscle remains largely unknown [5]. The biological effects of LPA are considered to be mediated by at least five GPCRs, designated as LPA1-5 [6-8]. The downstream effectors appear to be even more diverse and include activation of phospholipase C (PLC), tyrosine kinases and phosphatidylinositol 3-kinase (PI3-K) [9-13]. LPA receptors are known to be expressed in skeletal muscle cells and are considered to participate in the mitogenic effects and apoptosis [6, 14, 15]. In addition, LPA contained in serum has been reported to stimulate the proliferation and inhibit the differentiation of myoblast cells [15]; these actions of LPA were blocked by pertussis toxin (PTX), suggesting the involvement of Gi proteins in the LPA response [16].

Exogenous LPA can increase [Ca2+]i in different cell types [17-20] and has been reported to activate PI3-K in cardiomyocytes [21]. In addition, PI3-K has been demonstrated to regulate Ca2+ in T cells [17], suggesting a possibility that PI3-K might also be involved in mediating the LPA response in skeletal muscle.

In view of the critical importance of [Ca2+]i in the regulation of skeletal muscle function [22-25], the present study was therefore undertaken to examine if LPA can induce an increase in [Ca2+]i, to identify the sources of Ca2+ mobilization as well as to determine the signal transduction mechanisms that might be involved in mediating the LPA response in skeletal muscle C2C12 cells. Our results show for the first time that LPA can induce an increase in cytosolic and nuclear [Ca2+]i as well as DNA synthesis in skeletal muscle cells and myotubes, and may therefore play an important role in the regulation of [Ca2+]i and growth of skeletal muscle cells.

Materials and methods

Cell culture

C2C12 skeletal muscle cell line was obtained from the American Type Culture Collection (Manassas, VA, USA). The cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM, Invitrogen, ON, Canada) containing 10 % foetal bovine serum (FBS, Invitrogen, ON, Canada.) at 37°C with 95 % air and 5 % CO2. When the cells reached to 80 % of the confluence, they were incubated in DMEM containing 2 % FBS for a further 24 hrs prior to the experiments [26, 27].

Measurement of [Ca2+]i in C2C12 cell suspension

The LPA (L3-α-LPA, oleoyl (C18:1, [Cis]-9), Sigma-Aldrich, MO, USA)-induced changes in [Ca2+]i were measured according to methods previously described by Xu et al. [26]. Briefly, C2C12 cells were washed twice with serum free DMEM and then harvested with 0.25 % trypsin-1 mM ethylenediaminetetraacetic acid (EDTA). The cells were centrifuged at 240 g for 5 min. at room temperature. The supernatant was removed and the cells were washed two times in N-2-hydroxyethylpiperazine-N’ 2-ethane sulfonic acid (HEPES) buffer (pH 7.4) and were incubated for 40 min at 37°C with 10 µM fura-2/AM (Molecular Probes, OR, USA) in HEPES buffer. The cells were then washed twice with HEPES buffer to remove the extracellular dye and their number was adjusted to approximately 0.3 x 10^6 cells/ml. The cell suspension in the cuvette was stirred at the speed of 500 rpm. The fluorescence intensity of fura-2 was monitored by a SLM DMX-1100 dual-wavelength spectrofluorometer; the ratio (R) of fluorescence signal at 340/380 (nM) was calculated automatically. The Rmax and Rmin values were determined by the addition of 40 µl Triton X-100 (10%) and 20 µl ethylene glycol-bis (β-aminomethyl ether)-N, N', N'-tetraacetic acid (EGTA, 400 mM), respectively. The [Ca2+]i was calculated according to the following formula: [Ca2+]i = 224 x [(R-Rmin)/(Rmax-R)] x Sf2/Sb2, where Sf2 and Sb2 are the fluorescence proportionality coefficients obtained at 380 nm under Rmin and Rmax conditions, respectively [26, 27].

The pre-treatment of cells with the various pharmacological agents was performed by incubating cells in the buffer containing desired concentration of the different agents for 10 min at room temperature, except for the pre-treatment with LPA1/3 receptor antagonists, which was for 10 sec, prior to the measurement of fluorescence in response to LPA. In the experiments requiring pre-treatment with inhibitors, similar volume of vehicle, dimethyl sulfoxide (DMSO) or water, was added to the control group. The increase in [Ca2+]i at the peak transient was calculated as the net increase above the basal value in each experiment in response to LPA in the absence and presence of different interventions.

Determination of [Ca2+]i in single C2C12 cell

The measurement of [Ca2+]i in single cell was performed in confocal microscope (Nikon TE2000). The cells were cultured on the Glass Bottom Culture Dishes (MatTek, Ashland, MA, USA) for 6 days, and then the cells were loaded with 10 µM of fluo-3/AM in HEPES buffer for 30 min. After washing three times with HEPES buffer, the cells were
Assessment of DNA synthesis in C2C12 cells

The LPA-induced DNA synthesis in C2C12 cells was determined by measuring \[^{3}H\]-thymidine (Amersham, ON, Canada) incorporation by pulse labelling as described previously [27]. Cells in 12-well plates were serum starved for 20 hrs for synchronization, washed twice with serum-free medium and incubate with 0.5 ml serum-free medium. LPA was added in the presence of 100 nM wortmannin (Calbiochem, CA, USA) or 2 µl DMSO to control cells. After incubation for 4 hrs, 1 µCi \[^{3}H\]-thymidine was added. The reaction was stopped after 22 hrs by removing the medium on ice. The cells were washed three times with 1 ml HEPES buffer. These cells were then incubated for 1 hr in ice cold 5 % trichloroacetic acid (TCA) on the ice, washed two more times with 0.5 ml HEPES buffer, and incubated with 0.2 ml NaOH (0.5 N) for a period of 1 hr. The aliquots were transferred to scintillation vial. The radioactivity was counted in a Beckman LS 6500 scintillation counter after the addition of 10 ml scintillant (CytoScint, ICN, CA, USA).

Statistical analysis

The data are expressed as mean ± S.E.M. Statistical analysis was performed using the Microcal Origin Version 6 (Microcal Software Inc., MA, USA). The differences between two groups were evaluated by Student’s t-test. The data from more than two groups were evaluated by One-Way ANOVA followed by Student’s t-test for the comparison between control and treated group. The value of P less than 5 % (P < 0.05) was considered to be statistically significant.

Results

Concentration-dependent effect of LPA on \([\text{Ca}^{2+}]_i\) in C2C12 cells

We have previously reported that LPA increases \([\text{Ca}^{2+}]_i\) in A10 vascular smooth muscle cells (VSMCs) [26, 27]. Likewise, LPA (1–50 µM) induced a significant concentration-dependent increase in \([\text{Ca}^{2+}]_i\), with the maximum response at 30 sec that declined back to basal levels at 2 min (Fig. 1A). A maximum response was achieved at 50 µM LPA (Fig. 1A and B). A concentration of 10 µM LPA was subsequently used for the investigation of the mechanisms of the LPA-induced increase in \([\text{Ca}^{2+}]_i\). This was also based on other studies that have employed...
this concentration of LPA [21, 26, 27]. It should be noted 20 µl water served as a control and exerted no significant effect on basal [Ca^{2+}]; (data not shown).

Changes in [Ca^{2+}] in C2C12 cells in response to LPA in the presence and absence of LPA receptor antagonists and Gi protein inhibition

In order to determine if the LPA-induced increase in [Ca^{2+}] was a LPA-receptor mediated response, C2C12 cells were pre-treated with LPA_{1/3} receptor antagonists, dioctanoyl glycerol pyrophosphate (DGPP 8:0, 10 µM), VPC12249 (10 µM) or VPC 32183 (10 µM) [29–31] (Avanti Polar Lipids, Inc, Al, USA) for 10 sec before the addition of LPA. The pre-treatment with these receptor antagonists had no significant effect on the basal [Ca^{2+}] (data not shown). However, these receptor blockers significantly inhibited the LPA-induced increase in [Ca^{2+}]; (Fig. 2). Furthermore, to understand the LPA receptor signalling mechanisms, C2C12 cells were also pre-treated with a Gi protein inhibitor, pertussis toxin (PTX, 100 ng/ml, Sigma-Aldrich, MO, USA) [32] for 10 min. This time of pre-treatment with PTX is based on previous experience [21]. It can be seen in Figure 2 that the LPA-induced increase in [Ca^{2+}] in C2C12 cells was significantly inhibited.

Role of tyrosine kinase and phosphatidylinositols 3-kinases inhibitors in the LPA-induced increase in [Ca^{2+}] in C2C12 cells

To determine the role of tyrosine kinase on LPA-induced increase in [Ca^{2+}] in C2C12 cells, genistein (5 µM, Sigma-Aldrich, MO, USA) and tyrphostin A9 (A9, 5 µM, Sigma-Aldrich, MO, USA), tyrosine kinase inhibitors [33], were added 10 min prior to the addition of LPA. A significant attenuation of the LPA-induced Ca^{2+} response was seen by both inhibitors (Fig. 3A). The role of PI3-K in the LPA-induced increase in [Ca^{2+}] was examined by pre-treating C2C12 cells with the PI3-K inhibitors [17], wortmannin (100 nM) and LY 294002 (10 µM) for 10 min prior to addition of LPA. It can be seen in Figure 3B that both wortmannin and LY 294002 significantly attenuated the Ca^{2+} response to LPA by 57 % and 63 %, respectively. It should be noted that the pre-treatment the cells with genistine significantly reduced the basal [Ca^{2+}]; (vehicle control: 95±3; genistein: 63±3, P<0.05). Furthermore, pre-treatment with other agents had no significant effect on the basal [Ca^{2+}]; (vehicle control: 90±10 nM; A9: 85±11 nM; wortmannin: 80±6 nM; LY 294002 85±7 nM).

Ca^{2+} influx due to LPA

In C2C12 cells, EGTA (1 mM) reduced both the basal as well as the peak [Ca^{2+}]; response evoked by LPA, whereas verapamil and diltiazem had no significant effect on the basal and LPA-induced increase in [Ca^{2+}]; (Table 1). Similarly, both Gd^{3+} and La^{3+}, blockers of store operated Ca^{2+} channels (SOCs), did not exert any significant effect on LPA-evoked increase in [Ca^{2+}]; (Table 1). To test whether receptor operated Ca^{2+} channels (ROCs) play a role in LPA-induced changes in [Ca^{2+}]; in C2C12 cells, cells were pre-treated with SK&F 96365
(10 µM, Biomol Research Laboratory, PA, USA), an inhibitor of ROCs [34] and with the transient receptor potential (TRP) C7 cation channel blocker, 2-aminoethoxydiphenyl borate (2-APB, 100 µM, Sigma-Aldrich, MO, USA) [35] for 10 min. Both these agents significantly attenuated the LPA-induced increase in [Ca\(^{2+}\)](i) by 50–60 % (Fig. 4A). The cells pre-treated with SK&F 96365 and 2-APB did not alter the basal [Ca\(^{2+}\)](i) (vehicle control 81±8; SK&F 96365 81±11; 2-APB 100±12 nM). Pre-treatment with the Na\(^{+}\)–Ca\(^{2+}\) exchanger inhibitors, Ni (1 mM) and KB-R7943 (30 µM, Tocris Biosciences, MO, USA) [26], resulted in a significant inhibition (40–55%) of the LPA-induced increase in [Ca\(^{2+}\)](i) in C2C12 cells (Fig. 4B). The pre-treatment of cells with Ni and KB-R7943 alone exerted no significant action on the basal [Ca\(^{2+}\)](i) (vehicle control: 83±11 nM; Ni: 81±11 nM; KB-R7943: 91±12 nM).

**Sarcoplasmic reticulum (SR) Ca\(^{2+}\) release in response to LPA**

To determine the intracellular source for the LPA-induced increase in [Ca\(^{2+}\)](i), the following series of experiments were conducted. Pre-incubation of C2C12 cells with U73122 (1 µM, Sigma-Aldrich, MO, USA), a PLC inhibitor; xestospongin (XEC, 10 µM, Calbiochem, CA, USA), a membrane permeable inositol trisphosphate (IP\(_3\)) receptor blocker and thapsigargin (5 µM, Sigma-Aldrich, MO, USA), which depletes IP\(_3\) sensitive SR Ca\(^{2+}\) store [6], did not produce any significant inhibition on the LPA-evoked increase in [Ca\(^{2+}\)](i) (Table 2). The effect of 10 mM caffeine (final concentration) was also examined. As shown in Table 2, caffeine alone caused a significant increase in [Ca\(^{2+}\)](i), but it did not produce any effect on the LPA-induced increase in [Ca\(^{2+}\)](i).

**Table 1 Effect of different agents on Ca\(^{2+}\) influx into C2C12 skeletal muscle cells in response to exogenous LPA**

| Group          | Basal [Ca\(^{2+}\)](i) (nM) | LPA-induced increase in [Ca\(^{2+}\)](i) above the basal (nM) |
|----------------|-----------------|-----------------------------|
| Water (10 µl)  | 85 ± 15         | 76                          |
| EGTA (1 mM)    | 58 ± 6*         | 30 ± 6*                     |
| Verapamil (10 µM) | 96 ± 6       | 72 ± 15                     |
| Diltiazem (10 µM) | 95 ± 10        | 72 ± 8                      |
| Gd\(^{3+}\) (1 µM) | 94 ± 21        | 64 ± 6                      |
| La\(^{3+}\) (10 µM) | 98 ± 19        | 68 ± 10                     |

Values are means ± S.E.M. of six different measurements. C2C12 cells were pre-treated with EGTA for 30 sec, whereas pre-treatment with the other agents was for 10 min prior to addition of LPA (10 mM). The [Ca\(^{2+}\)](i) was measured as described in the Materials and methods. *P<0.05 versus vehicle control (water) group.
Although pre-treatment (30 sec) of the C2C12 cells with 30 mM KCl alone induced a small, but significant increase in the basal [Ca\textsuperscript{2+}]\textsubscript{i}, however, the LPA-induced increase of [Ca\textsuperscript{2+}]\textsubscript{i} that was inhibited by approximately 50% (Table 2).

### Localization of the increase in [Ca\textsuperscript{2+}]\textsubscript{i} in single C2C12 cell treated with LPA

Marius et al. [36] have reported that Ca\textsuperscript{2+} release from the nucleoplasmic reticulum (NR) plays an important role in the Ca\textsuperscript{2+} mobilization in C2C12 cells. To investigate whether LPA has an effect on Ca\textsuperscript{2+} concentration in nucleus, cells were loaded with fluo-3/AM and visualized by confocal microscopy. The fluo-3/AM dye was seen to be highly localized in the nucleus under resting conditions. This could be a reflection of either more [Ca\textsuperscript{2+}]\textsubscript{i} in the nucleus than in the cytosol or compartmentalization of the dye to the nucleus. It is pointed out that a similar observation has been reported in cultured cortical astrocytes [37]. Exogenous LPA caused a significant increase in the Ca\textsuperscript{2+} concentration in both the nucleus and cytoplasm (Fig. 5). The peak response was observed at 30 sec after addition of LPA.

### Effect of LPA on DNA synthesis in C2C12 cells

Intracellular Ca\textsuperscript{2+} signalling plays an important role in gene expression and DNA replication [36]. Since LPA has been observed to increase Ca\textsuperscript{2+} in both the nucleus and cytoplasm, it is possible that LPA might also induce DNA synthesis in C2C12 cells. Accordingly, we determined the effect of LPA on DNA synthesis in C2C12 cells. It can be seen in Figure 6 that LPA induced an increase in DNA synthesis as evidenced by an increase in [\textsuperscript{3}H]-thymidine incorporation in C2C12 cells, which was prevented by the PI3-K inhibitor, wortmannin (100 nM). Wortmannin alone had no effect on [\textsuperscript{3}H]-thymidine incorporation in C2C12 cells.

### Table 2 Effect of different agents on Ca\textsuperscript{2+} release due to LPA treatment of C2C12 skeletal muscle cells

| Group          | Basal [Ca\textsuperscript{2+}]\textsubscript{i} (nM) | LPA-induced increase in [Ca\textsuperscript{2+}]\textsubscript{i} above the basal (nM) |
|----------------|------------------------------------------------------|--------------------------------------------------------------------------------------|
| DMSO (10 µl)   | 93 ± 10                                               | 69 ± 11                                                                              |
| TG (5 µM)      | 195 ± 20*                                             | 63 ± 10                                                                              |
| Caffeine (10 mM)| 146 ± 12*                                             | 54 ± 10                                                                              |
| XEC (10 µM)    | 99 ± 8                                               | 66 ± 11                                                                              |
| U73122 (1 µM)  | 101 ± 5                                              | 75 ± 11                                                                              |
| KCl (30 mM)    | 123 ± 2*                                             | 36 ± 8*                                                                             |

Values are means ± S.E.M. of six different measurements. C2C12 cells were pre-treated with the different agents for 10 min prior to addition of LPA (10 mM). The [Ca\textsuperscript{2+}]\textsubscript{i} was measured as described in the Materials and methods. *P<0.05 versus vehicle control (DMSO) group. TG, thapsigargin; XEC, xestospongin.
Discussion

Several lines of evidence indicate that LPA is a lipid mediator, abundantly present in the blood, with a diverse range of biological actions including the regulation of \([\text{Ca}^{2+}]_i\) and proliferation in different cell types [2, 3, 17–20, 27, 38–41]. In fact, \(\text{Ca}^{2+}\) is considered to play a critical role in the activation of signal transduction mechanisms involved in cell proliferation [27]. However, there is no information in the literature regarding the effects of LPA on \([\text{Ca}^{2+}]_i\) in skeletal muscle. We are the first to report that LPA induces an increase in \([\text{Ca}^{2+}]_i\) in a concentration and time dependent manner in C2C12 cells. The LPA-induced increase in \([\text{Ca}^{2+}]_i\) was significantly inhibited by three LPA1/3 receptor antagonists, as well as with PTX, genistein and tyrphostin A9, suggesting that the LPA-induced increase in \([\text{Ca}^{2+}]_i\) is mediated by LPA1/3 receptors that may be coupled to G\(_i\) protein and tyrosine kinase (Fig. 7). Similar mechanisms of LPA-induced increase in \([\text{Ca}^{2+}]_i\) have also been reported in VSMCs and T cells [32, 33]. Exogenous LPA has also been reported to activate PI3-K in cardiomyocytes [21]. PI3-K has been demonstrated to

![Fig. 5](image-url) Lysophosphatidic acid-induced changes in nuclear and cytosolic calcium in single C2C12 cells. The C2C12 cells were loaded with 10 \(\mu\text{M}\) fluo-3/AM for 30 min. Cells were washed three times with HEPES buffer and scanned in time series with a Nikon TE2000-U confocal microscope. The \([\text{Ca}^{2+}]_i\) is reflected by the intensity of the green fluorescence of fluo-3.

![Fig. 6](image-url) Effect of lysophosphatidic acid on C2C12 cell proliferation in the absence and presence of phosphatidylinositol 3-kinase inhibition with wortmannin. The C2C12 cell proliferation was determined by the incorporation of \([\text{H}]\)-thymidine into C2C12 cells. 100 nM wortmannin (WOR) was added to the culture medium 10 min prior to the addition of LPA (10 \(\mu\text{M}\)). After a 4 hrs incubation with LPA, 1 \(\mu\text{Ci}\) \([\text{H}]\)-thymidine was added. The reaction was terminated after 22 hrs. Values are means \(\pm\) S.E.M. of six experiments. *\(P<0.05\) versus vehicle control value; #\(P<0.05\) versus LPA value in the absence of wortmannin.
regulate Ca\(^{2+}\) signalling in T cells [17]. In the present study, pre-treatment of C2C12 cells with PI 3K inhibitors wortmannin and LY 294002, significantly attenuated the Ca\(^{2+}\) response to LPA in C2C12 cells, indicating that PI3-K may be involved in Ca\(^{2+}\) mobilization in skeletal muscle. In view of the reported activation of PI3-K through Gi-protein in leukotriene \(\beta\)-induced degranulation in rat basophilic leukaemic cells [42], it is possible that the LPA\(_{1/3}\) receptor may transduce its signal to PI3-K via Gi-protein in skeletal muscle. However, an interaction between tyrosine kinase and PI3-K cannot be excluded and therefore warrants further investigation (Fig. 7). The partial inhibition observed with LPA\(_{1/3}\) receptor antagonists is suggestive of an involvement of other LPA receptors in the increase in [Ca\(^{2+}\)]\(\text{i}\) in response to LPA. Our preliminary data have revealed that higher concentrations of DGPP (50 and 100 \(\mu\)M) did not abolish the LPA-induced increase in [Ca\(^{2+}\)]\(\text{i}\), indicating that indeed other LPA receptor subtypes could be involved in the LPA response.

We have previously reported that EGTA significantly reduces the LPA-induced increase in [Ca\(^{2+}\)]\(\text{i}\) in VSMCs and have suggested that the LPA-induced increase in [Ca\(^{2+}\)]\(\text{i}\) may result from Ca\(^{2+}\) influx from extracellular space [26]. Furthermore, we have shown that the L-type Ca\(^{2+}\) channel blockers, verapamil and diltiazem, did not produce any significant change in the basal and LPA-mediated increase in [Ca\(^{2+}\)]\(\text{i}\) in VSMCs [26, 27]. Likewise, these blockers also had no significant effect on basal and LPA-induced increase in [Ca\(^{2+}\)]\(\text{i}\) in C2C12 cells. In addition, EGTA (1 mM) reduced both basal as well as peak [Ca\(^{2+}\)]\(\text{i}\) response evoked by LPA [Ca\(^{2+}\)]. Thus, similar to our earlier findings in VSMCs [26, 27], LPA does not appear to induce Ca\(^{2+}\) entry from the extracellular source through the L-type Ca\(^{2+}\) channels in skeletal muscle cells. LPA induced Ca\(^{2+}\) influx has been reported to occur via SOCs in VSMCs [26]. In the present investigation, SOCs blockers, Gd\(^{3+}\) and La\(^{3+}\) [25, 36] did not exert any significant effect on LPA-evoked increase in [Ca\(^{2+}\)]\(\text{i}\), indicating that Ca\(^{2+}\) mobilization by LPA does not involve extracellular Ca\(^{2+}\) entry through SOCs. A similar observation has also been reported in human neutrophils [20].

The role of Na\(^{+}\)–Ca\(^{2+}\) exchanger on LPA-induced increase in [Ca\(^{2+}\)]\(\text{i}\) in C2C12 cells was also investigated by pre-treating cells with two Na\(^{+}\)–Ca\(^{2+}\) exchanger inhibitors, Ni and KB-R7943 [26]. These two agents inhibited the LPA-initiated response by 40–55\%, indicating that LPA may induce Ca\(^{2+}\) influx through Na\(^{+}\)–Ca\(^{2+}\) exchanger. Similar results have been reported in VSMCs where an attenuation of the LPA-induced increase of [Ca\(^{2+}\)]\(\text{i}\) was observed in the presence of Na\(^{+}\)–Ca\(^{2+}\) exchanger inhibitors [26] suggesting that Ca\(^{2+}\) entry through Na\(^{+}\)–Ca\(^{2+}\) exchanger may be a common mechanism contributing to the LPA-induced increase [Ca\(^{2+}\)]\(\text{i}\) in both VSMCs and C2C12 skeletal muscle cells. Tokumura et al. [43] have reported that the LPA-induced activation of Na\(^{+}\)–Ca\(^{2+}\) exchanger is attenuated by tyrosine kinase inhibition in cultured bovine adrenal
chromaffin cells. In the present study, the increase of $[\text{Ca}^{2+}]_i$ induced by LPA in C2C12 cells was attenuated not only with $\text{Na}^+–\text{Ca}^{2+}$ exchanger inhibitors, but also with inhibition of tyrosine kinase activity. Thus, it would be interesting to examine if tyrosine kinase is involved in the regulation of $\text{Na}^+–\text{Ca}^{2+}$ exchanger activity in skeletal muscle cells (Fig. 7).

Although ROCs play an important role in $\text{Ca}^{2+}$ influx in T cells [44], their role in C2C12 cells have not been examined. Recently it has been reported that transient receptor potential (TRP) C7 cation channels are inhibited by 2-APB [44, 45]. In the present study pre-treatment of C2C12 cells, with SK&F 96365, a ROC blocker [39] and (TRP) C7 cation channels blocker 2-APB [42] significantly attenuated the LPA-induced increase in $[\text{Ca}^{2+}]_i$, by 50–60%. These results indicate that unlike that in VSMCs, where $\text{Ca}^{2+}$ mobilization by LPA involves extracellular $\text{Ca}^{2+}$ influx through SL SOCs, $\text{Ca}^{2+}$ mobilization from extracellular by LPA involves ROCs in skeletal muscle. It has been reported that ROCs are linked to G-proteins [47]. Furthermore, Su et al. [48] have shown that activation of PI3-K is involved in $\text{Ca}^{2+}$ dependent signalling at the level of ROCs in swine carotid arteries. Since ROCs have been reported to play an important role in $\text{Ca}^{2+}$ and that LPA activates PI3-K [12, 21], which is known to regulate $\text{Ca}^{2+}$ signalling in T cells [17], it is apparent that the LPA-induced intracellular $\text{Ca}^{2+}$ influx in skeletal muscle cells may involve ROCs through G-protein linked PI3-K ancillary to $\text{Na}^+–\text{Ca}^{2+}$ exchanger (Fig. 7).

Various studies have demonstrated that there are several $\text{Ca}^{2+}$ stores in the SR, and that different stimuli may cause $\text{Ca}^{2+}$ release from IP3-, caffeine- or KCl-sensitive SR stores [49–51]. Subsequent experiments were designed to delineate the source of intracellular $\text{Ca}^{2+}$ release contributing to the LPA-induced increase in $[\text{Ca}^{2+}]_i$ in C2C12 cells. While the LPA-induced increase in $[\text{Ca}^{2+}]_i$ in VSMCs has been shown to be mediated by PLC and IP$_3$ [26], a similar role for PLC and IP$_3$ in the LPA-evoked $[\text{Ca}^{2+}]_i$ response in C2C12 cells was not evident. In this study, no significant inhibition on the LPA evoked increase in $[\text{Ca}^{2+}]_i$ was found when C2C12 cells were pre-incubated with U73122, a PLC inhibitor and XEC, a membrane permeable IP$_3$ receptor blocker and thapsigargin a depletor of IP3 sensitive SR $\text{Ca}^{2+}$ store [26, 52]. These results suggest that an IP$_3$-dependent intracellular $\text{Ca}^{2+}$-store may not be involved in the increase in $[\text{Ca}^{2+}]_i$ due to LPA in C2C12 cells. While the LPA-induced increase in $[\text{Ca}^{2+}]_i$ in human neuroblastoma cells has also been reported to occur independently of IP3 or ryanodine receptors [53], the mechanism of LPA-induced increase in $[\text{Ca}^{2+}]_i$ in VSMCs involves the IP3-sensitive intracellular $\text{Ca}^{2+}$ pool and activation of PLC [26]. The vasopressin-induced $\text{Ca}^{2+}$ mobilization in neonatal rat cardiomyocytes has been reported to occur in a PLC-dependent manner and independent of the ryanodine- or caffeine-sensitive intracellular $\text{Ca}^{2+}$-stores, suggesting that there are two SR $\text{Ca}^{2+}$ pools, one a ryanodine-sensitive and the other an IP3- $\text{Ca}^{2+}$ sensitive pool [49]. In the present study, caffeine alone was found to cause a significant increase in $[\text{Ca}^{2+}]_i$, but it did not produce any effect on the LPA-induced increase in $[\text{Ca}^{2+}]_i$ in C2C12 cells. These data indicate that LPA may not induce $\text{Ca}^{2+}$ release from caffeine sensitive SR store.

Lorenzon et al. [54] have recently demonstrated that depolarization of C2C12 cells by KCl evokes $\text{Ca}^{2+}$ release from SR through non-caffeine-sensitive receptors, indicating the presence of voltage- and ligand-gated ryanodine receptors on SR in differentiated C2C12 cells. Some reports have also indicated that ryanodine receptor response to caffeine, voltage or ryanodine is functionally different [55, 56]. The effect of membrane depolarization by KCl in the LPA-induced response in C2C12 cells was also examined. Pre-treatment of C2C12 cells with 30 mM KCl alone induced a small, but significant increase in the basal $[\text{Ca}^{2+}]_i$; however, the LPA-induced increase of $[\text{Ca}^{2+}]_i$ was inhibited by about 50%. These data suggest that LPA may induce $\text{Ca}^{2+}$ release through voltage-gated ryanodine receptors from SR or NR. On the other hand, membrane depolarization by KCl is known to deplete SR $\text{Ca}^{2+}$ stores and suppress $\text{Ca}^{2+}$ entry [57] resulting in morphological and functional changes in the mitochondrial permeability transition pore [58], it is also possible that membrane depolarization by KCl may change the structural configuration of ROCs or $\text{Na}^+–\text{Ca}^{2+}$ exchanger and inactivate ROCs and $\text{Na}^+–\text{Ca}^{2+}$ exchanger. Although we have earlier reported a positive inotropic effect of LPA in rat heart in vivo [59], such a response in skeletal muscle is not known; however, in view of the critical importance of $[\text{Ca}^{2+}]_i$ in skeletal muscle contractile activity [22–25], it is likely that LPA would regulate a contractile response in skeletal muscle. Nevertheless, the functional
relevance of the LPA effects in C2C12 cells was addressed by assessment of DNA synthesis in response to LPA. We observed an increase in DNA synthesis as evidenced by the increase in [³H] thymidine incorporation due to LPA. Since wortmannin prevented the LPA-induced DNA synthesis in C2C12 cells, it can be suggested that PI3-K has an important role to play in LPA signal transduction mechanisms and skeletal muscle cell proliferation. In fact, the increase in nuclear Ca²⁺ in the C2C12 cells in response to LPA, observed in the present study, may serve as a signal for DNA synthesis [36] and thus may be related to cell proliferation. This aspect warrants further investigation.

**Conclusion**

Our findings suggest that LPA causes a time and concentration dependent increase in [Ca²⁺], which is dependent on extracellular Ca²⁺ concentration involving Ca²⁺ influx through Na⁺–Ca⁺ exchanger and ROCs in skeletal muscle cells but not SOCs as observed by Gutierrez-Martin et al. [60]. This action seems to be mediated through G-protein linked LPA₁₃ receptors, which in turn activates tyrosine kinase and PI3-K (Fig. 7). However, further studies are required to confirm the link between the tyrosine kinase and PI3-K with Na⁺–Ca⁺ exchanger and ROCs. The present study also demonstrated that the mechanisms of LPA-induced increase in [Ca²⁺] may depend upon cell type. The limitation of the present study includes the fact that the observations from cultured C2C12 cells may not represent the physiological response to LPA in skeletal muscle cells under in vivo conditions. Although the physiological role of the LPA-induced increase in [Ca²⁺] in C2C12 cells is remains to be completely understood, the results of the present study provide novel information on the mechanisms of LPA-induced increase in the cytosol and nuclear [Ca²⁺] as well as DNA synthesis in skeletal muscle cells. The increase in [Ca²⁺] due to LPA may serve as the trigger for the activation of signal transduction processes that ultimately result in cell proliferation. However, a direct relation between the increases in [Ca²⁺] and cell proliferation remains to be determined. Furthermore, the link between the changes in the nucleus versus cytosolic [Ca²⁺] also remains to be established. Nevertheless, our data suggest that LPA could play an important role in the regulation of skeletal muscle [Ca²⁺] and cell proliferation, which may lead to a novel treatment for skeletal muscle dystrophy.

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**References**

1. Mills GB, Moolenaar WH. The emerging role of lysophosphatidic acid in cancer. Nat Rev Cancer. 2003; 3: 582–91.
2. Xu Y-J, Aziz AA, Bhugra P, Arneja AS, Mendis MR, Dhalla NS. Potential role of lysophosphatidic acid in hypertension and atherosclerosis. Can J Cardiol. 2003; 19: 1525–38.
3. Swarouth T, Walling HW. Lysophosphatic acid: receptors, signaling and survival. Cell Mol Life Sci. 2000; 57: 1978-85.
4. Bockaert J, Pin JP. Molecular tinkering of G-protein-coupled receptors: an evolutionary success. EMBO J. 1999; 18: 1723–9.
5. Tang CM, Insel PA. GPCR expression in the heart; "new" receptors in myocytes and fibroblasts. Trends Cardiovasc Med. 2004; 14: 94–9.
6. Anliker B, Chun J. Cell surface receptors in lysophospholipid signaling. Semin Cell Dev Biol. 2004; 15: 457–65.
7. Hecht JH, Weiner JA, Post SR, Chun J. Ventricular zone gene-1 (vzg-1) encodes a lysophosphatic acid receptor expressed in neurogenic regions of the developing cerebral cortex. J Cell Biol. 1996; 135: 1071–83.
8. An S, Bleu T, Hallmark OG, Goetzi EJ. Characterization of a novel subtype of human G protein-coupled receptor for lysophosphatidic acid. J Biol Chem. 1998; 273: 7906–10.
9. Lee C-W, Rivera R, Gardell S, Dubin AE, Chun J. GPR92 as a new G₁₂/₁₃ and G₉ coupled lysophosphatic acid receptor that increases camp, LPA₅. J Biol Chem. 2006; 281: 23589–97.
10. van Corven EJ, Groenink A, Jalink K, Eicholtz T, Moolenaar WH. Lysophosphatidate-induced cell
proliferation: identification and dissection of signaling pathways mediated by G proteins. Cell. 1989; 59: 45–54.

11. van der Bend RL, de Widt J, van Corven EJ, Moolenaar WH, van Blitterswijk WJ. The biologically active phospholipid, lysophosphatic acid, induces phosphatidylcholine breakdown in fibroblasts via activation of phospholipase D. Comparison with the response to endothelin. Biochem J. 1992; 285: 235–40.

12. Moolenaar WH. Lysophosphatic acid, a multifunctional phospholipid messenger. J Biol Chem. 1995; 270: 12949–52.

13. Moolenaar WH, Kranenburg O, Postma FR, Xu Y-J, Ouk Kim S, Liao DF, Katz S, Pelech SL. Mechanisms of lysophosphatic acid-induced increase in intracellular calcium in vascular smooth muscle cells. Cell Calcium. 2005; 38: 569–79.

14. Jean-Baptiste G, Yang Z, Khoury C, Greenwood MT. Lysophosphatic acid mediates pleiotropic responses in skeletal muscle cells. Biochem Biophys Res Commun. 2003; 355: 115–62.

15. Yoshida S, Fujisawa-Sehara A, Taki T, Arai K, Nabeshima Y. Lysophosphatic acid and bFGF control different modes in proliferating myoblasts. J Cell Biol. 1996; 132: 181–93.

16. Tigyi G, Parrill AL. Molecular mechanisms of lysophosphatic acid action. Prog Lipid Res. 2003; 42: 498–526.

17. Eichholtz T, Jalink K, Fahrenfort I, Moolenaar WH. The bioactive phospholipid lysophosphatic acid is released from activated platelets. Biochem J. 1993; 291: 667–80.

18. Hsu AL, Ching TT, Sen G, Wang DS, Bondada S, Authi KS, Chen CS. Novel function of phosphoinositide 3-kinase in T cell Ca2+ signaling. A phosphatidylinositol 3,4,5-trisphosphate-mediated Ca2+ entry mechanism. J Biol Chem. 2000; 275: 16242–50.

19. Lee ZW, Kweon SM, Kim BC, Leem SH, Shin I, Kim JH, Ha KS. Phosphatidic acid-induced elevation of intracellular Ca2+ is mediated by RhoA and H2O2 in rat-2 fibroblasts. J Biol Chem. 1998; 273: 12710–5.

20. Itagaki K, Kannan KB, Hauser CJ. Lysophosphatic acid triggers calcium entry through a non-store-operated pathway in human neutrophils. J Leukoc Biol. 2005; 77: 181–9.

21. Xu Y-J, Ouk Kim S, Liao DF, Katz S, Pelech SL. Stimulation of 90- and 70 kDa ribosomal protein S6 kinases by arginine vasopressin and lysophosphatidic acid in rat cardiomyocytes. Biochem Pharmacol. 2000; 59: 1163–71.

22. Islam MN, Ochs RS. A new hypothesis for Ca2+ flows in skeletal muscle and its implications for other cell types. Cell Biochem Biophys. 2006; 44: 251–71.

23. Rome LC. Design and function of superfast muscles: new insights into physiology of skeletal muscle. Annu Rev Physiol. 2006; 68: 193–221.

24. Chin ER. Role of Ca2+/calmodulin-dependent kinases in skeletal muscle plasticity. J Appl Physiol. 2005; 99: 414–23.

25. Collet C, Pouvreaux S, Csernoche L, Allard B, Jacquemond V. Calcium signalling in isolated skeletal muscle fibres investigated under "Silicone Voltage-Clamp" conditions. Cell Biochem Biophys. 2004; 40: 225–36.

26. Xu Y-J, Saini HK, Cheema SK, Dhalla NS. Mechanisms of lysophosphatidic acid-induced increase in intracellular calcium in vascular smooth muscle cells. Cell Calcium. 2005; 38: 569–79.

27. Heinzle FR, Bito V, Volders PG, Antoons G, Mubagwa K, Sipido KR. Spatial and temporal inhomogeneities during Ca2+ release from the sarcoplasmic reticulum in pig ventricular myocytes. Circ Res. 2002; 91: 1023–30.

28. Fischer DJ, Nusser N, Virag T, Yokoyama K, Wang DA, Baker DL, Bautista D, Parrill AL, Tigyi G. Short-chain phosphatidates are subtype-selective antagonists of lysophosphatic acid receptors. Mol Pharmacol. 2001; 60: 776–84.

29. Park KS, Lee HY, Kim MK, Shin EH, Jo SH, Kim SD, Im DS, Bae YS. Lysophosphatidylycerine stimulates L2071 mouse fibroblast chemotactic migration via a process involving pertussis toxin-sensitive trimeric G-proteins. Mol Pharmacol. 2006; 69: 1066–73.

30. Heasley BH, Jarosez R, Lynch KR, Macdonald TL. Initial structure-activity relationships of lysophosphatic acid receptor antagonists: discovery of a high-affinity LPA1/LPA3 receptor antagonist. Bioorg Med Chem Lett. 2004; 14: 2735–40.

31. Seewald S, Sachinidis A, Dusing R, Ko Y, Seul C, Epping P, Vetter H. Lysophosphatic acid and intracellular signalling in vascular smooth muscle cells. Atherosclerosis. 1997; 130: 121–31.

32. Marhaba R, Mary F, Pelassy C, Stanescu AT, Ausssel C, Breitmayer JP. Tyrphostin A9 inhibits calcium release-dependent phosphorylations and calcium entry via calcium release-activated channel in Jurkat T cells. J Immunol. 1996; 157: 1468–73.

33. Facemire CS, Arendshorst WJ. Calmodulin mediates norepinephrine-induced receptor-operated calcium entry in pregglomerular resistance arteries. Am J Physiol Renal Physiol. 2005; 289: F127–36.
35. Lievremont JP, Numaga T, Vazquez G, Lemonnier L, Hara Y, Mori E, Trebak M, Moss SE, Bird GS, Mori Y, Putney JW Jr. The role of canonical transient receptor potential 7 in B-cell receptor-activated channels. J Biol Chem. 2005; 280: 35346–51.
36. Marius P, Guerra MT, Nathanson MH, Ehrlich BE, Leite MF. Calcium release from ryanodine receptors in the nucleoplasmic reticulum. Cell Calcium. 2006; 39: 65–73.
37. Zhao L, Brinton RD. Vasopressin-induced cytoplasmic and nuclear calcium signaling in cultured cortical astrocytes. Brain Res. 2002; 943: 117–31.
38. Goetzl EJ, Kong Y, Mei B. Lysosphatidic acid and sphingosine 1-phosphate protection of T cells from apoptosis in association with suppression of Bax. J Immunol. 1999; 162: 2049–56.
39. Sautin YY, Jorgensen M, Petersen BE, Saulnier-Blache JS, Crawford JM, Svetlov SI. Hepatic oval (stem) cell expression of endothelial differentiation gene receptors for lysosphatidic acid in mouse chronic liver injury. J Hematother Stem Cell Res. 2002; 11: 643–9.
40. Svetlov SI, Ignatova TN, Wang KK, Hayes RL, English D, Kukekov VG. Lysosphatidic acid induces clonal generation of mouse神经祖细胞 via proliferation of Sca-1- and AC133-positive neural progenitors. Stem Cells Dev. 2004; 13: 685–93.
41. Kim MK, Lee HY, Park KS, Shin EH, Jo SH, Yun J, Lee SW, Yoo YH, Lee YS, Baek SH, Bae YS. Lysosphatidic acid stimulates cell proliferation in rat chondrocytes. Biochim Pharmacol. 2005; 70: 1764–71.
42. Ito N, Yokomizo T, Sasaki T, Kurosu H, Penninger J, Kanaaho Y, Katada I, Hanaoka K, Shimizu T. Requirement of phosphatidylinositol 3-kinase activation and calcium influx for leukotriene B4-induced enzyme release. J Biol Chem. 2002; 277: 44898–904.
43. Tokumura A, Okuno M, Fukuzawa K, Houchi H, Tsuchiya K, Oka M. Positive and negative controls by protein kinases of sodium-dependent Ca2+ efflux from cultured bovine adrenal chromaffin cells stimulated by lysosphatidic acid. Biochim Biophys Acta. 1998; 1389: 67–75.
44. Rao GK, Kaminski NE. Induction of intracellular calcium elevation by Delta^2-tetrahydrocannabinol in T cells involves TRPC1 channels. J Leukoc Biol. 2006; 79: 202–13.
45. Lemonnier L, Trebak M, Lievremont JP, Bird GS, Putney JW Jr. Protection of TRPC7 cation channels from calcium inhibition by closely associated SERCA pumps. FASEB J. 2006; 20: 503–5.
46. Okada T, Inoue R, Yamazaki K, Maeda A, Kurosaki T, Yamakuni T, Tanaka I, Shimizu S, Ikenaka K, Imoto K, Mori Y. Molecular and functional characterization of a novel mouse transient receptor potential protein homologue TRP7. Ca2+-permeable cation channel that is constitutively activated and enhanced by stimulation of G protein-coupled receptor. J Biol Chem. 1999; 274: 27359–70.
47. Butta N, Uncely E, Gonzalez-Manchon C, Parrilla R, Ayuso MS. Pertussis toxin inhibition of alpha1-adrenergic or vasopressin-induced Ca2+ fluxes in rat liver. Selective inhibition of the alpha1-adrenergic receptor-coupled metabolic activation. J Biol Chem. 1993; 268: 6081–9.
48. Su X, Smolock EM, Marcel KN, Moreland RS. Phosphatidylinositol 3-kinase modulates vascular smooth muscle contraction by calcium and myosine light chain phosphorylation-independent and -dependent pathways. Am J Physiol Heart Circ Physiol. 2004; 286: H657–66.
49. Shi J, Mori E, Mori Y, Mori M, Li J, Ito Y, Inoue R. Multiple regulation by calcium of murine homologues of transient receptor potential proteins TRPC6 and TRPC7 expressed in HEK293 cells. J Physiol. 2004; 561: 415–32.
50. Liu P, Hopfner R, Xu YJ, Gopalakrishnan V. Vasopressin-evoked [Ca2+] responses in neonatal rat cardiomyocytes. J Cardiovasc Pharmacol. 1999; 34: 540–6.
51. McGeown JG. Interactions between inositol 1, 4, 5-trisphosphate receptor and ryanodine receptor in smooth muscle: one store of two? Cell Calcium. 2004; 35: 613–9.
52. Rondé P, Dougherty JJ, Nichols RA. Functional IP3- and ryanodine-sensitive calcium stores in presynaptic varicosities of NG108-15 (rodent neuroblastoma x glioma hybrid) cells. J Physiol. 2000; 529: 307–19.
53. Young KW, Bootman MD, Channing DR, Lipp P, Maycox PR, Meakin J, Challiss RA, Nahorski SR. Lysosphatidic acid-induced Ca2+ mobilization requires intracellular sphingosine 1-phosphate production. Potential involvement of endogenous EDG-4 receptors. J Biol Chem. 2000; 275: 38532–9.
54. Lorenzon P, Grohovaz F, Ruzzier F. Voltage- and ligand-gated ryanodine receptors are functionally separated in developing C2C12 mouse myotubes. J Physiol. 2000; 525: 499–507.
55. Gomez-Viquez L, Rueda A, Garcia U, Guerrero-Hernandez A. Complex effects of ryanodine on the sarcoplasmic reticulum Ca2+ levels in smooth muscle cells. Cell Calcium. 2005; 38: 121–30.
56. Shiromoka N, Rios E. Activation of Ca2+ release by caffeine and voltage in frog skeletal muscle. J Physiol. 1996; 493: 317–39.
57. Kurebayashi N, Ogawa Y. Depletion of Ca\textsuperscript{2+} in the sarcoplasmic reticulum stimulates Ca\textsuperscript{2+} entry into mouse skeletal muscle fibres. *J Physiol.* 2001; 533: 185–99.

58. Vrabec JP, Lieven CJ, Levin LA. Cell-type-specific opening of the retinal ganglion cell mitochondrial permeability transition pore. *Invest Ophthamol.* 2003; 44: 2774–82.

59. Xu Y-J, Rathi SS, Zhang M, Bhugra P, Dhalla NS. Mechanism of the positive inotropic effect of lysophosphatidic acid in rat heart. *J Cardiovasc Pharmacol Therap.* 2002; 7: 109–15.

60. Gutierrez-Martin Y, Martin-Romero FJ, Henao F. Store-operated calcium entry in differentiated C2C12 skeletal muscle cells. *Biochim Biophys Acta.* 2005; 1711: 33–40.