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Phospholipase A_2-derived lysophosphatidylcholine triggers Ca^{2+} entry in dystrophic skeletal muscle fibers

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ABSTRACT

Duchenne muscular dystrophy is an inherited disease caused by the absence of dystrophin, a structural protein normally located under the sarcolemma of skeletal muscle fibers. Muscle degeneration occurring in this disease is thought to be partly caused by increased Ca^{2+} entry through sarcolemmal cationic channels. Using the Mn^{2+} quench method, we show here that Mn^{2+} entry triggered by Ca^{2+} store depletion but not basal Mn^{2+} entry relies on Ca^{2+}-independent PLA_2 ($iPLA_2$) activity in dystrophic fibers isolated from a murine model of Duchenne muscular dystrophy, the *mdx*^{5cv} mouse. $iPLA_2$ was found to be localized in the vicinity of the sarcolemma and consistently, the $iPLA_2$ lipid product lysophosphatidylcholine was found to trigger Ca^{2+} entry through sarcolemmal channels, suggesting that it acts as an intracellular messenger responsible for store-operated channels opening in dystrophic fibers. Our results suggest that inhibition of $iPLA_2$ and lysophospholipid production may be of interest to reduce Ca^{2+} entry and subsequent degeneration of dystrophic muscle.

Introduction

Duchenne muscular dystrophy (DMD) is a severe disease caused by the lack of dystrophin, a 427 kDa protein located under the plasma membrane [1]. Degradation of dystrophic muscle fibers in DMD is likely to be partly caused by excessive and sustained Ca^{2+} entry through the sarcolemma, which leads to increased proteolysis and muscle injury [2–5]. In fibers lacking dystrophin, Ca^{2+} entry is increased at rest, due to enhanced spontaneous cationic channel activity, and is further increased during muscle activity [5–8]. Indeed, recent studies indicate that both store-operated channels activated upon Ca^{2+} store depletion and stretch-activated channels are involved in the enhanced Ca^{2+} entry occurring during dystrophic muscle activity [5,8].

Several hypotheses have been brought forward to explain the enhanced Ca^{2+} entry occurring at rest and during activity of dystrophic fibers. Local tears in the sarcolemma occurring during eccentric exercise may lead to local proteolytic activation of cationic channels, and result in increased Ca^{2+} entry [4]. The lack of dystrophin has also been proposed to be directly responsible for enhanced store-operated Ca^{2+} entry in dystrophic myotubes [9,10].

However, increased store-operated Ca^{2+} entry may also be explained by abnormal regulation of store-operated channels. Recent observations indicate that the sarcoplasmic reticulum Ca^{2+} sensor STIM1 is involved in the activation of store-operated channels, whose precise identity is still elusive but may involve Orai1 and/or members of the transient receptor potential channels (TRP) family in skeletal muscle [8,10,11]. Numerous reports also suggest that the Ca^{2+}-independent isoform of PLA_2 ($iPLA_2$) is involved in the regulation of store-operated channels, and that lysophospholipids produced by this enzyme trigger opening of store-operated channels [12–14].

In intact dystrophic fibers from *mdx*^{5cv} mice, we have recently demonstrated that store-operated Ca^{2+} entry is regulated by $iPLA_2$, and that the overexpression of this enzyme is likely to be responsible for the enhanced store-operated Ca^{2+} entry [15].

Using the Mn^{2+} quench method, we show here that $iPLA_2$ is selectively involved in the regulation of divalent cation entry triggered by Ca^{2+} store depletion in dystrophic fibers from *mdx*^{5cv} mice, as inhibition of its activity did not alter basal Mn^{2+} entry. In accordance with these findings, we show using Ca^{2+} imaging and the patch–clamp technique that the PLA_2 product lysophosphatidylcholine (LPC) triggered Ca^{2+} entry through cationic channels exhibiting the same pharmacology as store-operated channels. This suggests that increased local production of LPC by $iPLA_2$ in the vicinity of store-operated channels triggers Ca^{2+} entry

Abbreviations: DMD, Duchenne muscular dystrophy; $iPLA_2$, Ca^{2+}-independent phospholipase A_2; LPC, lysophosphatidylcholine; TRP, transient receptor potential channel

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through these channels in dystrophic fibers. Altogether these results provide new insights to the regulation of Ca^{2+} entry into dystrophic muscle, and suggest that inhibition of $iPLA_2$, lysophospholipid production and action may be of great interest to reduce Ca^{2+} entry and downstream degeneration of dystrophic muscle.

Materials and methods

Cell preparation. Dystrophic (*mdx*^{5cv}) mice (3–4 months old) were killed by cervical dislocation. Flexor Digitorum Brevis (FDB) muscles were removed quickly, and fibers were isolated as previously described [15]. *mdx*^{5cv} skeletal fibers were used from 18 to 28 h after isolation.

Ca^{2+} imaging and Mn^{2+} influx measurements. Intracellular Ca^{2+} concentration was monitored with the fluorescent Ca^{2+} indicator Fura-2AM (acetoxymethylester form of Fura-2, cell permeant), as previously described [15].

The Mn^{2+} quench technique was used to estimate divalent cation influx through the sarcolemma [7,15]. As Mn^{2+} has a similar permeability as Ca^{2+} through most plasma membrane Ca^{2+} channels, the quench of Fura-2 fluorescence when Fura-2 is excited at 360 nm allows estimation of Mn^{2+} entry through plasma membrane Ca^{2+} channels. Fibers were first loaded with Fura-2 as described above and $MnCl_2$ (100 μM) was added to the bath solution at the time indicated on records. As Mn^{2+} quenches Fura-2 fluorescence, Mn^{2+} influx through the sarcolemma triggers a decrease of the fluorescence of Fura-2 loaded cells excited at 360 nm (isobestic point of Fura-2). Records shown in Fig. 1 represent the decrease of Fura-2 fluorescence expressed as the % of F_{360} (initial fluorescence value of each single fiber was set to 100% after background subtraction). For average data, Mn^{2+} entry was measured as the maximal quench rate of Fura-2 fluorescence for each fiber (in %/min). To measure Mn^{2+} entry triggered by Ca^{2+} store depletion, thapsigargin (1 μM, an inhibitor of the sarcoplasmic reticulum Ca^{2+} ATPase) was added together with $MnCl_2$ (100 μM).

In order to avoid artifacts due to contraction, fibers were incubated with the myosin ATPase inhibitor *N*-benzyl-*p*-toluene sulfonamide (BTS, 30 μM) [16]. To avoid eventual influx through L-type voltage-gated Ca^{2+} channels, all experiments were performed in the presence of the L-type voltage-gated Ca^{2+} channel blocker nifedipine (1 μM). Ca^{2+} transients or Mn^{2+} quench were measured in the whole perimeter of fibers. All experiments were carried out at room temperature (22 °C).

LPC or arachidonic acid was quickly applied to single cells by pressure ejection using a pinch valve pressurized perfusion system (ALA Scientific Instruments, USA) connected to a quartz micromanifold, with an output tip size of 100 μm. The micromanifold was mounted on a Leitz micromanipulator, to stimulate individual skeletal muscle fibers for the period indicated on records.

Immuno-staining. Isolated FDB fibers were stored in 8-well chamber slides (Lab-Tek) coated with Matrigel (400 μg/ml, Collaborative Research). Fibers were fixed in PBS with 1.5% formaldehyde and permeabilized with 0.2% Triton X-100. After extensive washing, non-specific binding sites were blocked with goat serum. Fibers were incubated overnight at 4 °C with the rabbit anti-$iPLA_2$ primary antibody (1/500, Cayman, USA). After extensive washing, fibers were incubated with anti-rabbit secondary antibody (1/1000) coupled to Alexa Fluor 488 for 1 h at room temperature. Fibers were mounted in Mowiol before observation with a Leica SP2 confocal microscope (equipped with a 40× oil immersion lens).

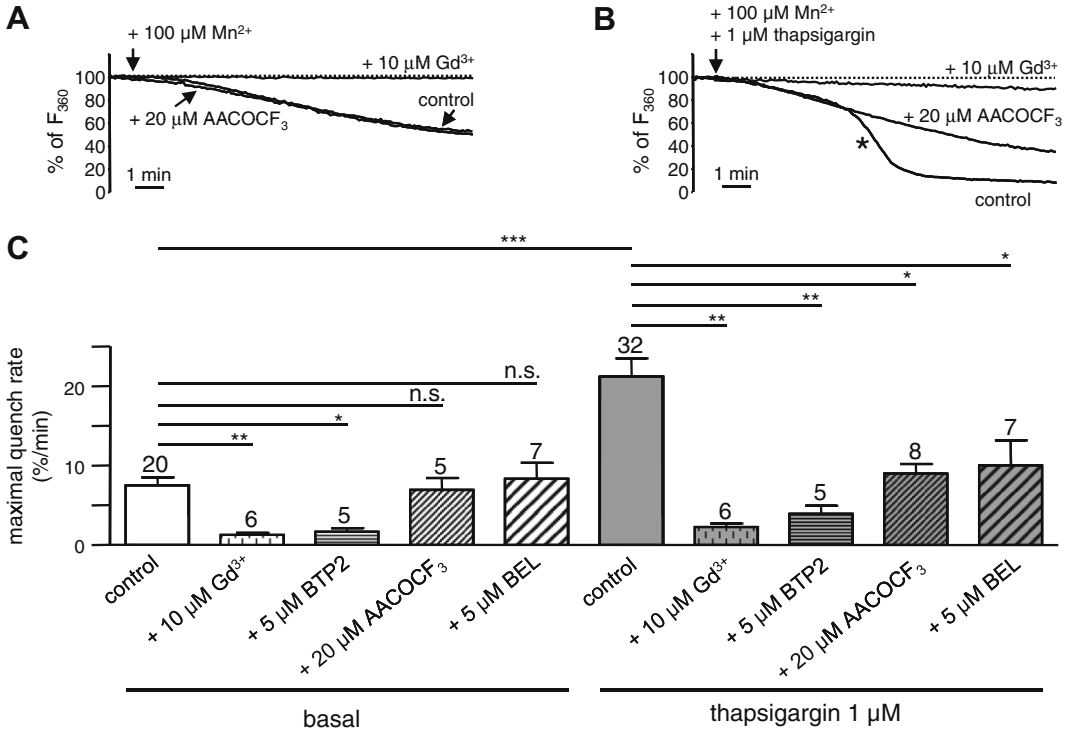

Fig. 1. Effect of cationic channel blockers and PLA_2 inhibitors on Mn^{2+} entry at rest and during Ca^{2+} store depletion in dystrophic (*mdx*^{5cv}) fibers. (A) Basal Mn^{2+} entry (expressed as the % of fluorescence for an excitation wavelength of 360 nm) following addition of 100 μM $MnCl_2$ in control and after preincubation with Gd^{3+} (10 μM, 5 min preincubation) or $AACOCF_3$ (20 μM, 10 min preincubation). (B) Mn^{2+} entry in *mdx*^{5cv} fibers following addition of 1 μM thapsigargin and 100 μM $MnCl_2$ in control and after preincubation with Gd^{3+} (10 μM, 5 min preincubation) or $AACOCF_3$ (20 μM, 10 min preincubation). The star indicates the delayed accelerated Mn^{2+} entry for the control experiment. (C) Plot of average maximal quench rates (in %/min) recorded upon Mn^{2+} or thapsigargin/Mn^{2+} addition in control conditions and in the presence of Gd^{3+}, BTP2 (5 μM, 10 min preincubation), $AACOCF_3$ and BEL (5 μM, 20 min preincubation). Number of fibers tested (from five *mdx*^{5cv} mice) are indicated on top of the bars.

Patch–clamp recordings. Patch–clamp recordings were performed in excised inside-out patches at room temperature obtained on individual fibers isolated as described above. Fibers were immersed in a high KCl solution (in mM: 10 NaCl, 142 KCl, 2 $MgCl_2$, 0.2 $CaCl_2$, 5 glucose, 0.5 EGTA, 10 Hepes; pH 7.3) mimicking the intracellular salt composition with 2 μg/ml of cytochalasin D added to inhibit stretch-activated channels excitation by rearranging actin microfilaments upon patch excision [17]. The tested agents, 20 μM LPC and 100 μM arachidonic acid were added to this solution and cells were perfused utilizing a pinch valve free flow perfusion system (Bioscience tools, San Diego, CA, USA). In order to avoid fiber contractions induced by transferring them into high KCl solution, they were treated with 30 μM BTS for 15 min before being transferred into the recording chamber [16]. Patch pipettes were pulled from borosilicate glass WPI 1B100F-4 (World Precision Instruments, Stevenage, UK) on a horizontal puller P2000 (Sutter Instruments, Novato, CA, USA) and filled with a $CaCl_2$ solution (110 mM $CaCl_2$, 10 mM Hepes; pH 7.3) with 2 mM tetraethyl ammonium (TEA) and 20 μM 4,4′-diisothiocyanato-stilbene-2,2′-disulfonic acid (DIDS) added to inhibit K^+ and Cl^- currents. Under these conditions patch pipette resistances were in the range of 3–4 MΩ. After establishing the gigaohm seal and excising the patch into an inside-out configuration, 30 mV were applied to the outer surface of the patch membrane. Inward Ca^{2+} currents were acquired in a gap free mode and low-pass filtered at 1 kHz with a Multiclamp 700B amplifier and then digitized with a Digidata 1322 digitizer (Molecular Devices, Sunnyvale, CA, USA) at 5 kHz.

Chemicals. Arachidonyltrifluoromethyl ketone ($AACOCF_3$), [*N*-(4-[3,5-bis(trifluoromethyl)-1*H*-pyrazol-1-yl]phenyl)-4-methyl-1,2,3-thiadiazole-5-carboxamide] (BTP2), *N*-benzyl-*p*-toluene-sulfonamide (BTS) and thapsigargin were from Calbiochem. Collagenase type IA, bromoenol lactone (BEL) and lysophosphatidylcholine (LPC) were from Sigma. Fura-2AM was from Molecular Probes. Ethylene glycol-bis(2-aminoethyl)-*N*,*N*,*N*′,*N*′-tetra acetic acid (EGTA), tetraethyl ammonium (TEA) and 4,4′-diisothiocyanato-stilbene-2,2′-disulfonic acid (DIDS) were from Fluka.

Statistics. Results are expressed as means ± SEM. Significance was tested by means of Student's *t* test and *p* values of <0.05 were considered as significant.

Results

The Mn^{2+} quench method, Ca^{2+} imaging and patch–clamp measurements were used to investigate the role of $iPLA_2$ and its metabolites in regulating activity of cationic channels at rest and after Ca^{2+} store depletion in dystrophic (mdx^{5cv}) fibers.

Effect of $iPLA_2$ inhibition on Mn^{2+} entry at rest and upon Ca^{2+} store depletion

Mn^{2+} entry at rest and following Ca^{2+} store depletion was measured in dystrophic (mdx^{5cv}) fibers using the Mn^{2+} quench method [7,15]. Fig. 1A shows a typical decrease of Fura-2 fluorescence due to basal Mn^{2+} entry, after addition of 100 μM Mn^{2+} to bath solution. Basal Mn^{2+} entry was strongly reduced when fibers were incubated with the cationic channel blockers Gd^{3+} or BTP2 [18] (from 7.48 ± 1.03%/min in control to 1.24 ± 0.28%/min and 1.66 ± 0.45%/min for Gd^{3+} and BTP2, respectively, Fig. 1A and C), indicating that these blockers inhibit basal Mn^{2+} influx occurring through cationic channels.

Thapsigargin, an inhibitor of the sarcoplasmic reticulum Ca^{2+} ATPase, was used to deplete Ca^{2+} stores, triggering opening of store-operated channels. When Mn^{2+} and thapsigargin were added together, Mn^{2+} entry was strongly accelerated (star in Fig. 1B) in comparison to basal Mn^{2+} entry (7.48 ± 1.03%/min and 21.2 ± 2.33%/min for Mn^{2+} entry at rest and upon Ca^{2+} store depletion, respectively, Fig. 1B and C). The delayed acceleration of Mn^{2+} entry in Fig. 1B can be explained by the requirement of significant Ca^{2+} store depletion before opening of store-operated channels can occur. As observed with basal Mn^{2+} entry, it was abolished when fibers were treated with the channel blockers Gd^{3+} or BTP2 (2.23 ± 0.45%/min and 3.9 ± 1.03%/min for Gd^{3+} and BTP2, respectively, Fig. 1B and C).

Preincubation of dystrophic fibers with $AACOCF_3$, a PLA_2 inhibitor blocking both Ca^{2+}-dependent and Ca^{2+}-independent PLA_2 [19,20] did not affect basal Mn^{2+} entry (6.9 ± 1.52%/min, Fig. 1A and C), but strongly reduced the enhanced Mn^{2+} entry of thapsigargin-treated fibers (8.97 ± 1.23%/min, Fig. 1B and C). Similar results were obtained when fibers were pretreated with the $iPLA_2$ specific suicide substrate bromoenol lactone (BEL, Fig. 1C) [19,20]. Altogether, these results indicate that $iPLA_2$ is responsible for the enhanced Mn^{2+} entry occurring upon Ca^{2+} store depletion, suggesting a specific localization for this enzyme and also that $iPLA_2$ hydrolysis products are involved in the gating of store-operated channels.

Immuno-localization of $iPLA_2$ dystrophic fibers

We performed immuno-staining experiments using a specific anti-$iPLA_2$ antibody and confocal microscopy. Fig. 2 shows a representative confocal section of a dystrophic fiber stained for $iPLA_2$. When the primary antibody was omitted, no fluorescence was detected, indicating that $iPLA_2$ staining did not result from non-specific binding of the secondary antibody. In the thin confocal section presented in Fig. 2 (0.5 μM thickness), $iPLA_2$ appears to be primarily located in the vicinity of the sarcolemma.

Effect of LPC and arachidonic acid on $[Ca^{2+}]_i$ in dystrophic fibers

$iPLA_2$ are enzymes that catalyze the hydrolysis of fatty acid ester bonds at the second position of diacylglycerophospholipids, leading to the release of arachidonic acid and lysophospholipids [20]. Both lysophospholipids and arachidonic acid metabolites have been shown to be activators of various cationic channels [12–14,21–25]. Recent evidence suggests that lysophospholipids, such as LPC produced by $iPLA_2$, can stimulate opening of cationic channels including store-operated channels [12–14,23–25]. To investigate which of these PLA_2 products may be responsible for store-operated channel activation, we tested the effect of external application of LPC and arachidonic acid on dystrophic fibers. External application of LPC triggered slow $[Ca^{2+}]_i$ increases of 72.4 ± 7.8 nM (Fig. 3A and F) while arachidonic acid had no effect on $[Ca^{2+}]_i$ ($n = 7$, Fig. 3A). LPC-induced $[Ca^{2+}]_i$ increases were strongly reduced by incubating fibers in Ca^{2+}-free solution

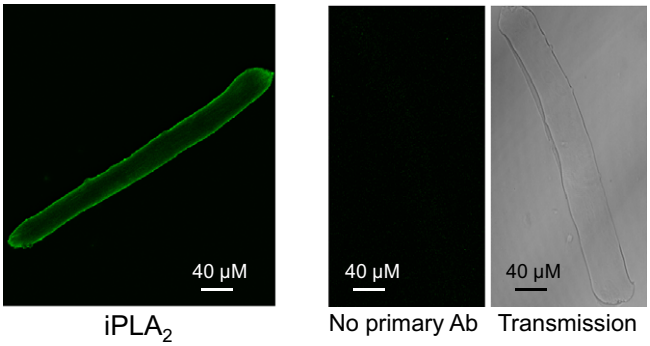

Fig. 2. Immuno-localization of $iPLA_2$ in mdx^{5cv} fibers. Left: Confocal section of a mdx^{5cv} fiber immuno-stained for $iPLA_2$. Right: when the primary antibody was omitted, no fluorescence was detected (control).

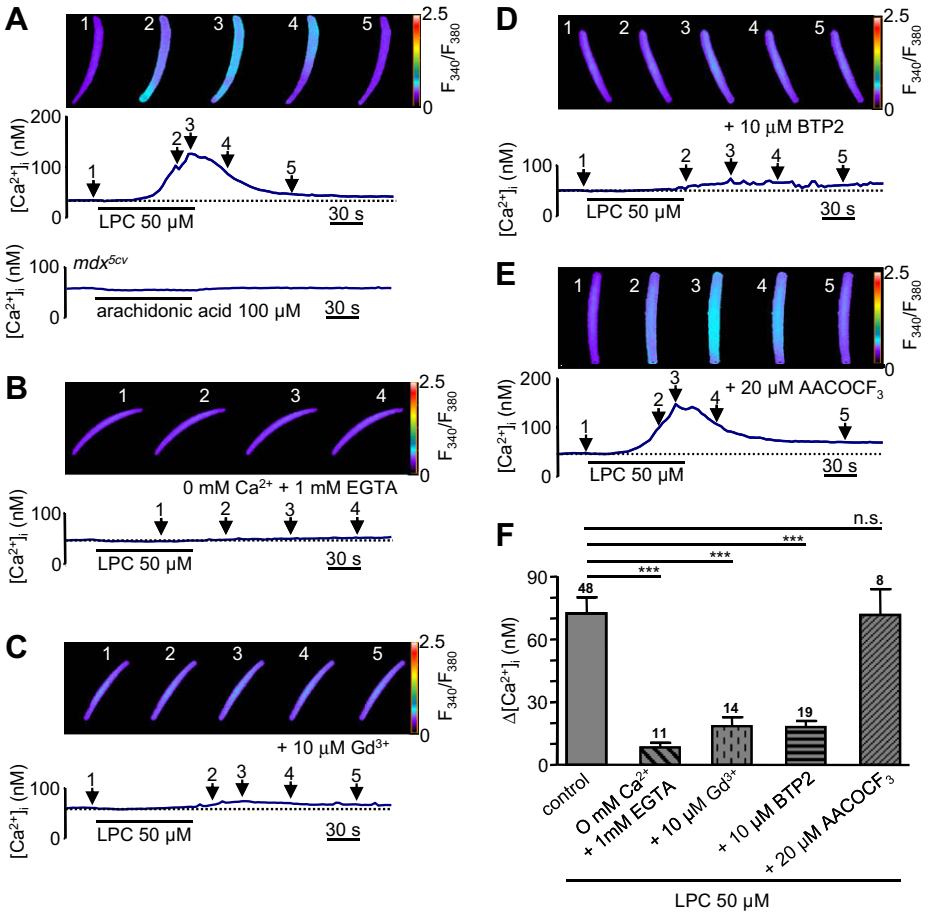

Fig. 3. Effect of the PLA_2 products LPC and arachidonic acid on cytosolic Ca^{2+} levels in *mdx*^{5cv} fibers. (A) Effect of external application of LPC (50 μM) and arachidonic acid (100 μM) on $[Ca^{2+}]_i$ in *mdx*^{5cv} fibers. Top panel shows time series of pseudocolor F_{340}/F_{380} ratio images corresponding to LPC-induced $[Ca^{2+}]_i$ increase. (B) Effect of LPC on $[Ca^{2+}]_i$ in Ca^{2+}-free solution containing 1 mM EGTA. (C) Effect of LPC on $[Ca^{2+}]_i$ in a *mdx*^{5cv} fiber preincubated for 5 min with 10 μM Gd^{3+}. (D) Effect of LPC on $[Ca^{2+}]_i$ in a *mdx*^{5cv} fiber preincubated for 10 min with 10 μM BTP2. (E) Effect of LPC on $[Ca^{2+}]_i$ in a *mdx*^{5cv} fiber preincubated for 10 min with 20 μM $AACOCF_3$. (F) Average values showing the effect of the absence of external Ca^{2+}, Gd^{3+}, BTP2 and $AACOCF_3$ on LPC-induced Ca^{2+} transients. Numbers of fibers tested (from five *mdx*^{5cv} mice) are indicated on top of the bars.

(8.2 ± 2.4 nM, Fig. 3B and F), indicating that the LPC effect on $[Ca^{2+}]_i$ was mainly due to Ca^{2+} entry through the sarcolemma. Involvement of L-type voltage-gated Ca^{2+} channels in LPC-induced Ca^{2+} entry can be discarded as all experiments were performed in the continuous presence of the L-type voltage-gated Ca^{2+} channel blocker nifedipine (1 μM). LPC-induced Ca^{2+} entry was significantly reduced by preincubating fibers with either Gd^{3+} or BTP2 (18.4 ± 4.3 and 18 ± 2.9 nM for Gd^{3+}- and BTP2-treated fibers, respectively, Fig. 3C, D and F), indicating that LPC-induced Ca^{2+} entry is mainly due to stimulation of cationic channels but not to unspecific effects. Since LPC may increase PLA_2 activity in some cases [26], we tested the effect of PLA_2 inhibition on LPC-induced Ca^{2+} entry. Incubation of dystrophic fibers with $AACOCF_3$ did not affect LPC-induced Ca^{2+} entry (71.5 ± 12.5 nM, Fig. 3E and F). To investigate if LPC-induced Ca^{2+} entry could be caused by stimulation of receptors coupled to phospholipase C such as lysophosphatidic acid receptors [27], fibers were pretreated with the phospholipase C inhibitor U73122 [28]. Such pretreatment did not have any inhibitory effect on LPC-induced Ca^{2+} entry (n = 12, not shown), indicating that the main phospholipase C products (inositol 1,4,5-trisphosphate and diacylglycerol) are not involved in the LPC effect.

Effect of LPC and arachidonic acid on Ca^{2+} currents in dystrophic fibers

In order to investigate whether the external nature of application of LPC and arachidonic acid could have affected the action of these agents, we used the patch–clamp technique to measure their effects on Ca^{2+} currents in excised inside-out patches. Fig. 4 shows typical currents elicited by perfusing these patches with bath solution containing either LPC (20 μM) or arachidonic acid (100 μM). While LPC reliably stimulated Ca^{2+} currents, significantly augmenting basal activity (n = 5 patches, Fig. 4A), arachidonic acid did not produce any noticeable current increase (n = 5 patches, Fig. 4B).

Discussion

In this study, we have investigated the role of $iPLA_2$ and its metabolites in the regulation of cationic channels active at rest and during Ca^{2+} store depletion in dystrophic (*mdx*^{5cv}) muscle fibers.

Basal and store-operated Mn^{2+} entry were strongly reduced by the general cation channel blocker Gd^{3+} and by BTP2, a compound described as a store-operated channel blocker [18]. This indicates that both basal and the accelerated Mn^{2+} entry triggered by Ca^{2+} store depletion are related to the opening of channels exhibiting similar pharmacology, possibly TRP channels and, more likely Orai1, as described in skeletal muscle [8,10,11]. Inhibition of $iPLA_2$ activity abolished the accelerated Mn^{2+} entry triggered by Ca^{2+} store depletion, in accordance with our previous study, while basal Mn^{2+} entry was not affected [15]. This indicates that $iPLA_2$ is causing activation of store-operated Mn^{2+} entry in a specific manner, and therefore that basal divalent cation entry is not under the control of $iPLA_2$ in dystrophic fibers. Mechanisms linking Ca^{2+} store

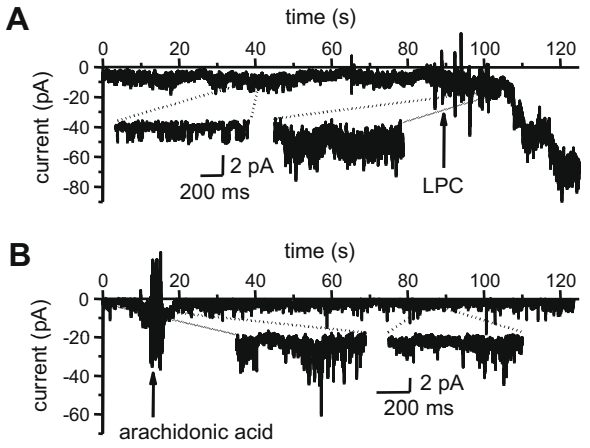

Fig. 4. LPC and arachidonic acid effects on Ca^{2+} current in excised inside-out patches. (A) Basal activity, commonly observed in *mdx*^{5cv} fibers, is significantly augmented upon addition of 20 μM LPC. Insets show an expanded view of the regions of the overall trace indicated by dotted lines on a scale allowing visualizing single channel openings before (left) and after (right) LPC addition. (B) Treatment of the excised patches with 100 μM arachidonic acid does not produce any significant effect. Both panels show typical long-term responses (overall curve) as well as expanded views allowing to distinguish single-channel activity before (left inset) and after (right inset) LPC or arachidonic acid application.

depletion and $iPLA_2$ stimulation remain elusive, but the sarcoplasmic reticulum Ca^{2+} sensor STIM1 may trigger the production of a yet unknown soluble messenger called Calcium Influx Factor (CIF), which triggers dissociation of the $iPLA_2$-calmodulin complex, leading to desinhibition of $iPLA_2$ [12].

The role of $iPLA_2$ in the regulation of store-operated channels suggests a specific localization of $iPLA_2$ in dystrophic fibers. $iPLA_2$ was found to be mainly localized in the vicinity of the sarcolemma, suggesting a close proximity with cationic channels such as store-operated channels, which may be located on the sarcolemma and/or in the T-tubular membranes.

The involvement of $iPLA_2$ in store-operated Mn^{2+} entry also suggests that a lipid product of $iPLA_2$ is responsible for the opening of store-operated channels in dystrophic fibers. $iPLA_2$ catalyzes the hydrolysis of fatty acid ester bonds at the second position of diacylglycerophospholipids, leading to the release of arachidonic acid and lysophospholipids [20]. Both lysophospholipids and arachidonic acid metabolites have been shown to be potent activators of channels such as store-operated or TRP/cationic channels [12–14,21–25]. In dystrophic fibers, we only found the PLA_2 product LPC to be effective in triggering Ca^{2+} entry, while arachidonic acid was found to have no noticeable effect on Ca^{2+} and sarcolemmal channel activity. LPC triggered Ca^{2+} entry through Gd^{3+}- and BTP2-sensitive channels, demonstrating that LPC-activated channels exhibit similar pharmacology as store-operated channels. LPC-induced Ca^{2+} entry was not affected by PLA_2 inhibition, indicating that LPC acts downstream of $iPLA_2$ and directly activates sarcolemmal channels. Using patch–clamp recording of cation channel activity in inside-out patches, we also demonstrate that LPC but not arachidonic acid activates large macroscopic Ca^{2+} currents, indicating that LPC is a powerful activator of sarcolemmal channels and that it is effective when applied at the inner face of the sarcolemma. This effect of LPC may be related to the direct activation of sarcolemmal channels, or to the modification of the lipid bilayer structure due to increased local LPC concentration, which may be sensed by channels such as TRP channels [23,24].

Altogether, our findings indicate that LPC produced by $iPLA_2$ plays a major role in the control of Ca^{2+} entry occurring upon Ca^{2+} store depletion in dystrophic fibers, a phenomenon that occurs during muscle activity [15,29]. Enhanced Ca^{2+} entry through cationic channels at rest or stimulated by Ca^{2+} store depletion or stretch of the sarcolemma is thought to be an important trigger for degeneration of dystrophic muscle, due to the Ca^{2+}-dependent activation of proteolytic enzymes [2–5,8]. Therefore, inhibition of $iPLA_2$, lysophospholipid production or action may be of great benefit to protect dystrophic fibers from excessive Ca^{2+} entry. Moreover, this may also be beneficial in reducing the oxidative stress and inflammation, two phenomena contributing to muscle degeneration that may be enhanced by PLA_2-derived lipids [30,31].

In summary, our results suggest that LPC produced by $iPLA_2$ may act locally as a messenger for activation of store-operated channels. Upon Ca^{2+} store depletion, increased $iPLA_2$ expression [15] and activity may lead to increased production of LPC in the vicinity of store-operated channels in dystrophic fibers, causing opening of these channels. As excessive Ca^{2+} influx through such channels is thought to be partly involved in the degeneration of dystrophic muscle, $iPLA_2$ may constitute a new interesting target for the treatment of DMD.

Acknowledgments

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