Induction of ornithine decarboxylase in T/C-28a2 chondrocytes by lysophosphatidic acid: Signaling pathway and inhibition of cell proliferation

Annalisa Facchini[a], Rosa Maria Borzì[b], Flavio Flamigni[a,*]

[a] *Dipartimento di Biochimica "G. Moruzzi", University of Bologna, Via Irnerio 48, 40126 Bologna, Italy*
[b] *Laboratorio di Immunologia e Genetica, Istituto di Ricerca Codivilla Putti, I.O.R., Via di Barbiano 1/10, 40136 Bologna, Italy*

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Abstract Among several extracellular messengers tested, lysophosphatidic acid (LPA) was able to cause the most marked induction of ornithine decarboxylase (ODC) in serum-starved human T/C-28a2 chondrocytes. LPA also induced the activation/phosphorylation of Src, Akt and p44/42 MAPK, and the translocation of PKC-δ from cytosol to membrane coupled to its tyrosine phosphorylation. Experiments with selective signaling inhibitors indicate that LPA leads to Src activation through Gi protein-coupled receptors. In turn Src can activate PI3K and PKC-δ, and all these signaling proteins are required for ODC induction. In conclusion these results show that chondrocytes may be a novel target for LPA action. However, although LPA is considered a mitogen for several cell types and ODC induction is generally correlated to cell growth, LPA was not able to stimulate chondrocyte growth, but rather exerted an anti-proliferative effect.

Keywords: Chondrocytes; Lysophosphatidic acid; Ornithine decarboxylase; Signal transduction; Cell proliferation

1. Introduction

Chondrocytes, the only cellular component of articular cartilage, differentiate and proliferate in human beings during the development, then, for the rest of adult life, they may be regarded as terminally differentiated cells, which are responsible for the synthesis, metabolic control and integrity of cartilage extracellular matrix [1]. However, in articular diseases, such as osteoarthritis, chondrocytes may be diverted from their homeostasis and undergo cellular alterations such as apoptosis, dedifferentiation, proliferation, hypertrophy and phenotype modulation resulting in the synthesis of embryonal-type matrix components and secretion of matrix degrading enzymes. External stimuli, including soluble factors such as cytokines, chemokines and growth factors [2,3] can influence the cellular and biochemical functions of chondrocytes, by regulating signaling pathways and gene expression. However, the extracellular stimuli and intracellular pathways that can play a role in various physiological or pathological contexts are only partially known.

Ornithine decarboxylase (ODC), the first and rate-limiting enzyme of polyamine synthesis [4], is induced in a cell type-specific way by a large variety of external stimuli, particularly those leading to cell growth. ODC and polyamines are well known to be essential for cell proliferation, but may also be involved in other cellular responses, such as differentiation and apoptosis [5]. Although little is known about the function and regulation of ODC in chondrocytes, some studies have reported high polyamines levels in rheumatoid arthritis [6] and the effectiveness of ODC inhibitors to avoid experimental arthritis induced in mouse [7]. Furthermore, we have found quite recently [8] that ODC inhibition reduces the chondrocyte response to TNF, a cytokine believed to play a critical role in arthritis. This background prompted us to investigate possible extracellular stimuli and signal transduction pathways leading to the induction of ODC in human T/C-28a2 chondrocytes [9,10], which have been reported to mimic native articular chondrocytes in primary culture [11]. We have found in particular that lysophosphatidic acid (1-oleyl-sn-glycerol 3-phosphate, LPA) can induce a marked ODC induction and the activation of key signaling proteins in these cells, but these responses do not appear to be related to chondrocyte proliferation.

2. Materials and methods

2.1. Materials

Anti-phospho specific and total Src antibodies were purchased from Biosource, whereas an anti phospho-tyrosine antibody, which recognizes general tyrosine-phosphorylated peptides, was obtained from Cell Signaling Technology/New England Biolabs (p-Tyr-102, cat. #9416, Lot.1). Anti-phospho specific (Thr202/Tyr204) and total p44/42 MAPK, anti-phospho specific (Ser473) and total Akt were also from New England Biolabs, anti-PKC-δ from Santa Cruz and anti-Ki-67 from Oncogene. Bacterial lipopolysaccharide (LPS), ATP, sphingosylphosphorylcholine (SPC), LPA, sphingosine-1-phosphate (SPP), phorbol myristate acetate (PMA), isoproterenol, parathyroid hormone (PTH) and pertussis toxin (PTX) were obtained from Sigma.

*Corresponding author. Fax: +39 051 2091224.
E-mail address: flavio.flamigni@unibo.it (F. Flamigni).

Abbreviations: bFGF, fibroblast growth factor-basic; FCS, fetal calf serum; IGF-I, insulin-like growth factor-I; LPA, lysophosphatidic acid; LPS, bacterial lipopolysaccharide; MAPK, mitogen-activated protein kinase; ODC, ornithine decarboxylase; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; PMA, phorbol myristate acetate; PTH, parathyroid hormone; PTX, pertussis toxin; SPC, sphingosylphosphorylcholine; SPP, sphingosine-1-phosphate; TGF-β1, transforming growth factor-β1; TNF, tumor necrosis factor-α

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PP1, LY 294002, PD 98059, U0126, BAPTA/AM and rottlerin were from Alexis. Tumor necrosis factor-α (TNF) and transforming growth factor-β1 (TGF-β1) were from Cabru, human recombinant insulin-like growth factor-I (IGF-I) and fibroblast growth factor-basic (bFGF) from Peprotech; green tea extract (GTE) was from Indena and histamine from RBI. DMEM, fetal calf serum (FCS), antibiotics and L-glutamine were purchased from Cambrex S.r.L.

2.2. Cell culture and treatment

The immortalized human juvenile costal chondrocytes, T/C-28a2 [9,10], have been used extensively as a reproducible "in vitro" model to study a variety of chondrocyte responses in experiments requiring large numbers of cells. T/C-28a2 chondrocytes were grown in DMEM with 10% FCS, 2 mM L-glutamine and antibiotics (100 U/ml penicillin, 100 μg/ml streptomycin). For experiments confluent cells were kept in a serum-free medium for 24 h before treatment with LPA or other compounds. All the inhibitors were added 30 min before LPA, except PTX (4 h before). These compounds were added in dimethylsulfoxide (except PTX solubilized in water). Control cells received equal amounts of the vehicle. LPA was solubilized in a solution $CHCl_3$:CH_3OH (2:1) and then frozen in aliquots at −20 °C. Each aliquot was exsiccated under nitrogen and the pellet was resuspended in the same volume of PBS-BSA (0.4%, fatty acid free). This solution was stable for 24 h. Each experiment was repeated at least twice. Graphs refer to experiments with at least duplicate samples and immunoblots show one representative result.

2.3. Determination of ODC activity

Cell extracts were prepared and assayed for ODC activity as previously described [12]. ODC activity is expressed as units/mg protein, where 1 unit corresponds to 1 nmol of CO_2/h incubation.

2.4. Immunoprecipitation and detection of phosphorylated PKC-δ

Cells were treated with LPA for the time indicated and then harvested in lysis buffer containing 20 mM HEPES, 2 mM EDTA, 0.1% CHAPS, 0.1% Triton X-100, 5 mM DTT, 1 mM PMSF, 1 μg/μl of aprotinin, leupeptin and pepstatin, 1 mM Na_3VO_4, 1 mM benzamidine, 10 mM PNPP, 10 mM β-glycerophosphate. Aliquots corresponding to 500 μg proteins (in the case of Fig. 5B) or 200 μg proteins (in the case of Fig. 5C) were incubated with antibody against PKC-δ for 2 h at 4 °C and then with protein A–Sepharose for a further 1 h. The immunocomplex was centrifuged, washed three times with lysis buffer and dissolved in Laemmli buffer. Immunoprecipitated proteins were separated in 10% SDS–PAGE, transferred on to nitrocellulose membrane. The membrane was saturated with 5% non-fat milk and probed with anti phospho-tyrosine antibody overnight at 4 °C. Immunoreactivity was detected with the ECL system and, in the case of the experiment shown in Fig 5C, quantified by intensitometric software QScan from Biosoft.

2.5. Western blotting for p60 Src, Akt, Ki-67 and p44/42 MAPK

Cells were incubated and harvested as described before. As for p60 Src, Akt and Ki-67, aliquots of 80 μg proteins were separated by SDS–PAGE and transferred on to nitrocellulose membrane. The membrane was saturated with 5% non-fat milk and then probed with specific antibodies overnight at 4 °C (90 min at R.T. in the case of Ki-67). Bands were revealed by the Amersham ECL detection system. Western blotting for p44/42 MAPK was performed as described [13].

2.6. Subcellular fractionation and detection of PKC-δ

Cells were incubated and harvested with lysis buffer containing 250 mM sucrose, 2 mM EGTA, 1 mM EDTA, 1 mM PMSF, 20 mM HEPES. Homogenisation with 45 strokes was carried out at 4 °C. Homogenates were centrifuged for 10 min at 500*g* at 4 °C and the resulting supernatant was centrifuged for 30 min at 110000*g*. The resulting pellet was resuspended in the same buffer containing 1% Triton X-100 and protease inhibitors (leupeptin, pepstatin, and aprotinin) and centrifuged for 50 min at 110000*g*. The supernatant (Triton X-100 soluble membrane fraction) was stored at −80 °C. 50 μg proteins of cytosol and membrane fraction were dissolved in Laemmli buffer, separated by 10% SDS–PAGE and transferred onto nitrocellulose membrane. The membrane was saturated with 5% non-fat milk and then incubated with anti PKC-δ antibody. Bands were revealed by the Amersham ECL detection system.

2.7. Cell proliferation assay

Cells were seeded at low density (5000 cells/well) in microtiter plates. After 1 day cells were shifted to a serum-free medium and treated with LPA or serum. The number of cells was evaluated by the cell proliferation kit I (MTT) obtained from Boehringer–Mannheim. This colorimetric assay is based on the cleavage of the yellow tetrazolium salt MTT to purple formazan crystals by metabolic active cells and was carried out according to Manufacturer's instructions. Cell viability was evaluated by Trypan blue exclusion, and expressed as the percentage of viable cells (excluding the dye) with respect to the total number of cells. In some experiments (Fig. 6B), the number of cells was evaluated by means of the PicoGreen dsDNA quantitation reagent (Molecular Probes, Eugene, OR), constructing a calibration curve with known number of chondrocytes, as detailed elsewhere [8]. Caspase activity was also assayed as described in [8].

3. Results

3.1. Effect of various extracellular stimuli on ODC activity in T/C-28a2 chondrocytes

In order to identify some possible agents able to induce ODC activity in chondrocytes, we tested a large number of extracellular stimuli. Most of these stimuli may play a role in chondrocyte biology or have been found to induce ODC in other cell types [14–16]. Apart from 10% fetal calf serum, a universal and strong ODC inducer, IGF-I, which may promote both proliferation and differentiation of chondrocytes [17], and LPA, which in contrast has no reported role in chondrocyte biology, were able to cause a substantial induction of ODC activity (Fig. 1). In particular, LPA, a ligand of specific G protein-coupled receptors [18–20], provoked an increase by about 10-fold on average. Other lysophospholipids that may act as extracellular messengers, i.e. SPC and SPP, and TNF, which can favour apoptosis in chondrocytes [21], caused a more modest increase. Finally, other messengers, including PTH, TGF-β, stromal cell-derived factor-1 (SDF-1) and bFGF were ineffective in these cells. Thus the study was focused on LPA effects. The time-course and dose-dependence of ODC activity are shown in Fig. 2. After an initial lag period, ODC activity in-

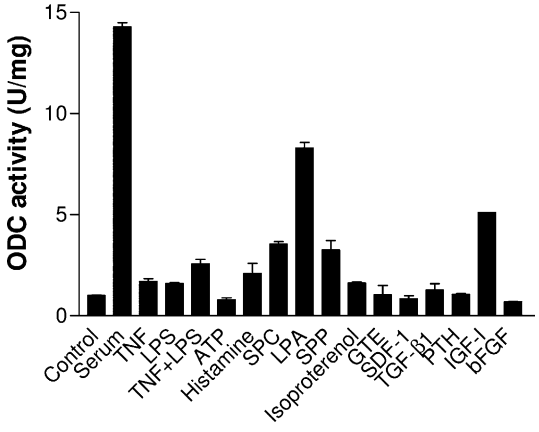

Fig. 1. Effect of various extracellular stimuli on the induction of ODC activity in T/C-28a2 chondrocytes. Confluent, serum-starved T/C-28a2 chondrocytes were treated with the indicated compounds. After 4 h cells were harvested and assayed for ODC activity. Concentrations of compounds in cell medium was as follows: fetal calf serum, 10%; TNF, 500 U/ml; LPS, 10 μg/ml; ATP, 100 μM; histamine, 10 μM; SPC, 10 μM; LPA, 10 μM; SPP, 10 μM; isoproterenol, 10 μM; GTE, 50 μM; SDF-1, 100 nM; TGF-β1, 10 ng/ml; PTH, 100 nM; IGF-I, 10 nM; bFGF, 5 ng/ml. Data are means ± S.E.M.

creased with a broad peak from 4 to 8 h following the addition of 10 μM LPA, then the enzyme activity declined and was close to the control value after 16 h (Fig. 2A). The dose-effect graph shows that the induction of ODC activity was markedly increased at 10 μM LPA and remained elevated even at 20 μM, but decreased using a higher concentration of the compound (Fig. 2B).

3.2. Effect of inhibitors of key signaling proteins on ODC induction

We have assessed the relevance of key signaling proteins in LPA-stimulated chondrocytes, such as Gi proteins, p60 Src tyrosine kinase, protein kinase C-δ (PKC-δ), p44/42 mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) by using selective inhibitors. The effects of PTX (Gi protein inhibitor), PP1 (p60 Src tyrosine kinase inhibitor), rottlerin (PKC-δ inhibitor), LY 294002 (PI3K inhibitor) and PD 98059 (p44/42 MAPK pathway inhibitor) on ODC induction are shown in Fig. 3. Although p44/42 MAPK activation has been reported to be involved in ODC induction provoked by various agents in several cell types [13–16,22] and LPA actually caused an increase in the amount of active/phosphorylated MAPK in T/C-28a2 cells (Fig. 3A), experiments with the specific inhibitor PD 98059 indicated that this kinase is not required for ODC induction by LPA in these cells (Fig. 3B). U0126, another selective and even more potent inhibitor of the MAPK pathway, could not prevent the ODC increase either (not shown). Instead, PTX was able to cause a dramatic reduction of the induction of ODC activity in our experimental model, suggesting that Gi proteins are involved in the signal

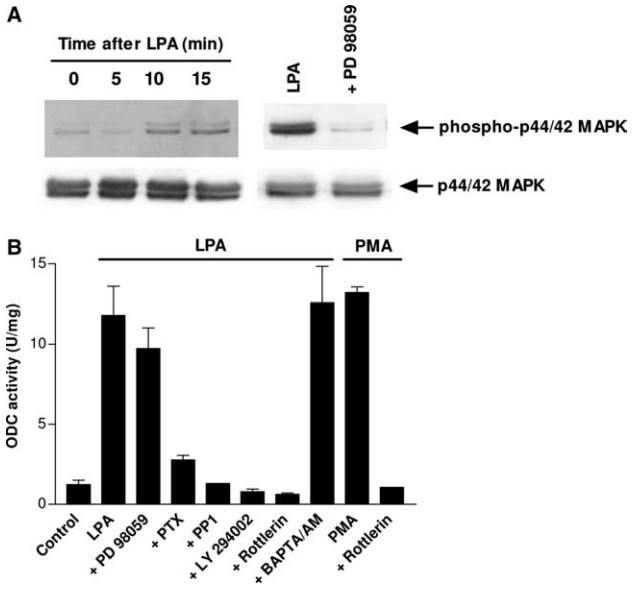

Fig. 3. Effect of LPA on p44/42 MAPK (A) and of signaling inhibitors on ODC induction (B). (A) Confluent, serum-starved cells were incubated with 10 μM LPA for the time indicated and then analysed for active, phosphorylated p44/42 MAPK by Western blotting. The membrane was then stripped and re-probed with a total anti-p44/42 MAPK antibody. In a distinct experiment (right), PD 98059 was also added before LPA to check its effectiveness in inhibiting MAPK activation. (B) Confluent, serum-starved cells were treated with the indicated inhibitors 30 min (4 h in the case of PTX) before treatment with 10 μM LPA or 100 ng/ml PMA. After 4 h cells were harvested and assayed for ODC activity. Data are means ± S.E.M.

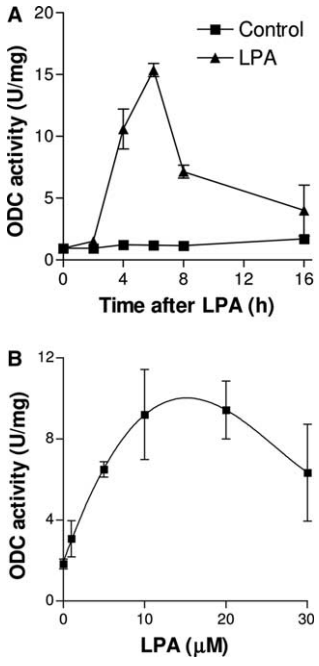

Fig. 2. Time course and dose-dependence of ODC induction by LPA in T/C-28a2 cells. (A) Effect of LPA on the time-course of ODC activity. 10 μM LPA was administered to confluent, serum-starved cells. At the time indicated cells were harvested and assayed for ODC activity. (B) Effect of increasing concentration of LPA on ODC activity. Confluent, serum-starved cells were treated with LPA at the indicated concentration, harvested after 4 h and assayed for ODC activity. Data are means ± S.E.M.

transduction pathway leading to the induction of ODC activity. Also PP1, LY 294002 and rottlerin were effective to inhibit the induction of ODC activity, suggesting the involvement of p60 Src tyrosine kinase, PI3K and PKC-δ, respectively. PKC-δ, like any other "novel" PKC isoform, does not depend on intracellular Ca^{2+} levels, but can be activated by phorbol esters, such as PMA. Thus the involvement of PKC-δ was also suggested by the lack of effect of BAPTA/AM, an intracellular Ca^{2+} chelator, together with the ODC inducing effect of PMA, which was likewise prevented by rottlerin (Fig. 3B).

3.3. Effect of LPA on Src and Akt activation

The previous experiments with PP1 prompted us to investigate whether LPA was able to activate Src. Src has a complex mechanism of activation, which comprises the autophosphorylation of a specific tyrosine residue (Tyr416) and the dephosphorylation of another one [23]. In our experimental conditions we found that LPA increased the phosphorylation of Src at Tyr416 within 5 min of treatment (Fig. 4A). It has been reported that in certain conditions the complete activation of Src also requires the dephosphorylation of Tyr527 [23]. However this dephosphorylation is not always needed [24] and in our conditions, LPA did not reduce the phosphorylation of Tyr527 (data not shown). We tested the effect of the inhibitors, which were previously shown to be effective in reducing ODC activity. PP1 was used as a positive control. PTX reduced the increase in the phosphorylation of Src, in accordance with many studies that indicate that Gi proteins are involved in the signal transduction of LPA from the membrane receptor to Src [18]. Although it has been reported by

Lopez-Illasaca et al. [24] that Src is a mediator of PI3K, in our conditions, LY 294002 was unable to modify the phosphorylation state of Src, suggesting that PI3K may rather lay downstream of Src or on a separate pathway. Rottlerin was not able to reduce the enhanced phosphorylation of Src by LPA either, indicating that PKC-δ is not required for this phosphorylation. To understand if PI3K lies downstream of Src or on a separate pathway, we evaluated the effect of the Src inhibitor, PP1, on the activation state of a typical substrate of PI3K, Akt (Fig. 4B). LPA was able to activate Akt by increasing its phosphorylation in a PI3K-dependent manner as shown by the sensitivity to LY 294002. PP1 abolished this activation indicating that Src is upstream of PI3K, as described in other experimental systems [23,25].

3.4. Effect of LPA on PKC-δ activation

Since the previous experiments suggested the involvement of PKC-δ in the action of LPA, we tested the effect of LPA on the activation of this protein and investigated if PKC-δ lays downstream Src in our experimental model. The translocation from cytosol to the membrane is a well-established mechanism of activation of PKC-δ [26–29]. Phosphorylation of PKC-δ on tyrosine residues can be a further mechanism of modulation of the enzyme, often resulting in an increase in its kinase activity, depending on cellular type and substrate involved [26–30]. In chondrocytes, LPA induced both the translocation and the phosphorylation of PKC-δ (Fig. 5A and B), suggesting that even in our experimental model PKC-δ tyrosine phosphorylation is coupled to an activation of the enzyme. We also tested the effect of the signaling inhibitors on the phosphorylation status of PKC-δ (Fig. 5C). All the compounds (LY 294002, PP1, Rottlerin and PTX) were effective in reducing the phos-

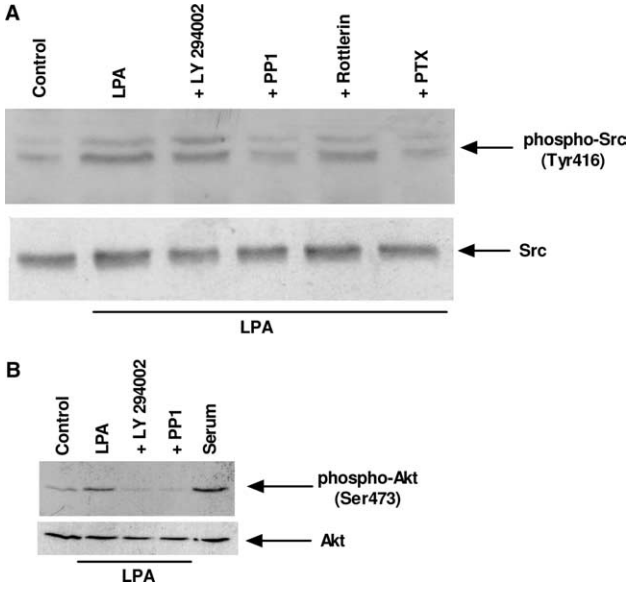

Fig. 4. LPA activates Src and Akt kinases in T/C-28a2 cells. (A) LPA induces the activation of p60 Src kinase by increasing the phosphorylation of a specific tyrosine residue (Tyr416). Confluent, serum-starved cells were incubated with the indicated inhibitors 30 min before the addition of 10 μM LPA (4 h in the case of PTX). After 5 min cells were harvested and cell extracts were analyzed by Western blotting by using a phospho-specific Src (Tyr416) antibody. The membrane was stripped and re-probed with a total anti-Src antibody. (B) LPA induces the activation of Akt in T/C-28a2 cells, by increasing its phosphorylation. Confluent, serum-starved cells were incubated with 10 μM LPA for 5 min and with the indicated inhibitors 30 min before the addition of LPA. Cells were harvested and cell extracts were analyzed by Western blotting by using a phospho-specific Akt (Ser473) antibody. The membrane was stripped and re-probed with a total anti-Akt antibody.

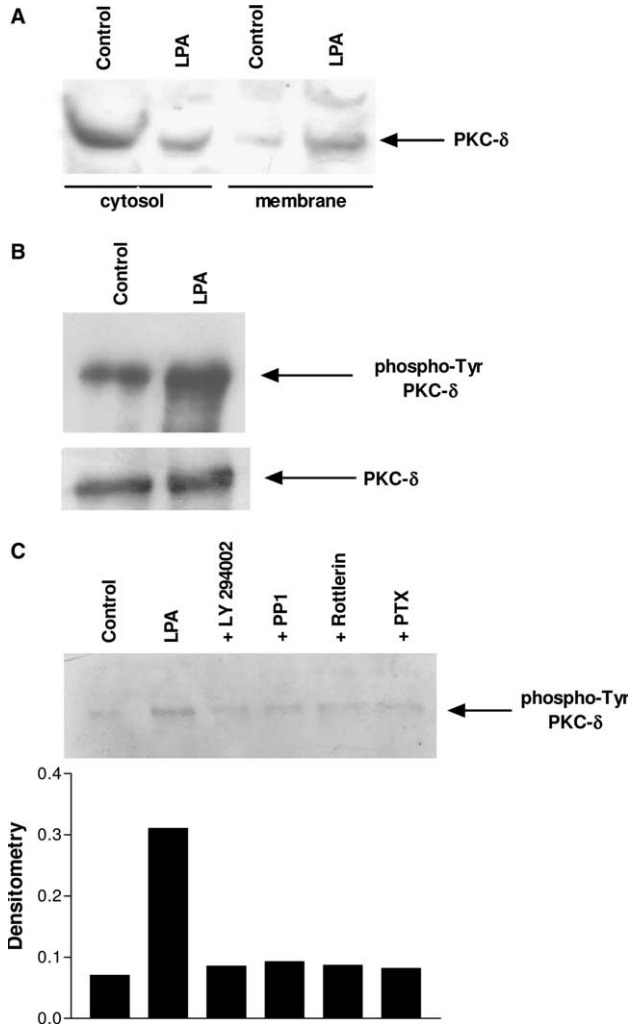

Fig. 5. LPA activates PKC-δ in T/C-28a2 cells. (A) LPA induces the translocation of PKC-δ from cytosol to membrane. Confluent, serum-starved cells were treated with 10 μM LPA. After 5 min cells were harvested and subcellular fractions were analyzed by Western blotting using a specific anti PKC-δ antibody. (B) LPA induces tyrosine phosphorylation of PKC-δ. Confluent, serum-starved cells were incubated with 10 μM LPA. After 5 min cells were harvested and cell extracts immunoprecipitated with anti-PKC-δ antibody. The immunocomplexes were analyzed by Western blotting by using a general phospho-tyrosine antibody. The membrane was stripped and re-probed with an anti-PKC-δ antibody. (C) Various signaling inhibitors prevent the LPA-induced tyrosine phosphorylation of PKC-δ. Confluent, serum-starved T/C-28a2 cells, were incubated with the indicated inhibitors 30 min before the addition of 10 μM LPA (4 h in the case of PTX). PKC-δ tyrosine phosphorylation was detected as described in the legend of Fig. 5B, except for the lesser starting material used for immunoprecipitation (see Section 2), which accounts for the weaker intensity of the bands of Fig. 5C compared to Fig. 5B. The membrane was stripped and re-probed with an anti PKC-δ antibody resulting in minimal differences among bands (not shown). Quantification by densitometry of phospho-Tyr PKC-δ normalized for total PKC-δ is shown at the bottom.

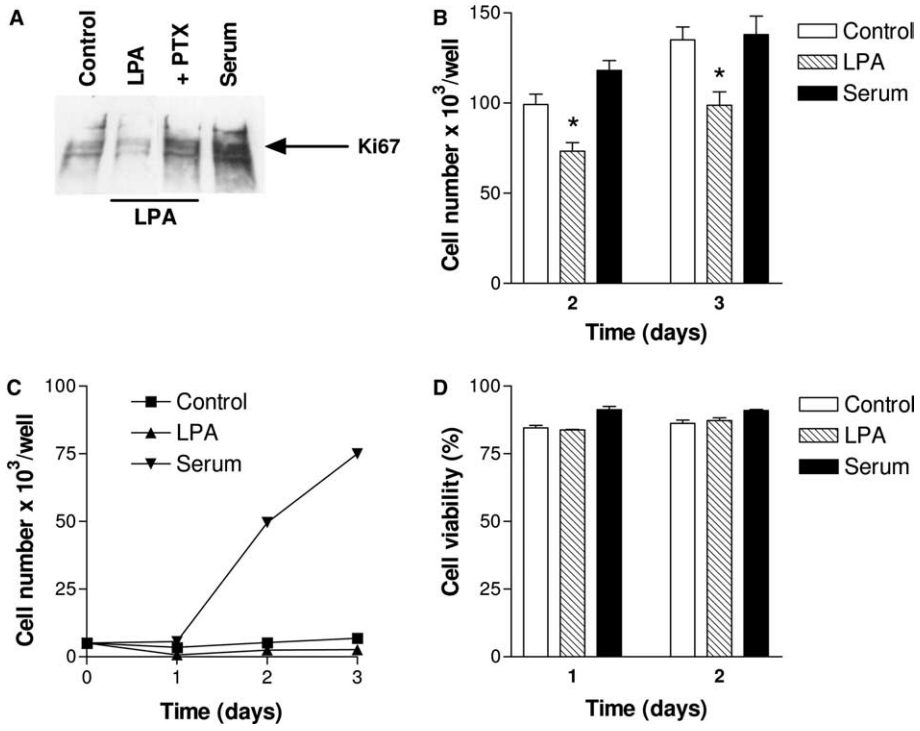

Fig. 6. Effect of LPA and serum on Ki-67 expression and cell proliferation and viability. (A, B) Confluent, serum-starved cells were incubated with 10 μM LPA or 10% serum. Ki-67 level (A) was determined in cell extracts by Western blotting after 20 h incubation. Cell number (B) was determined by DNA quantitation after 2 and 3 days of incubation. Data are means ± S.E.M., $N = 3$. $^{*}P < 0.05$ vs. control by unpaired t test. (C, D) Non-confluent cell cultures were incubated with 10 μM LPA or 10% serum as described in Section 2. Cell proliferation (C) and cell viability (D) were evaluated by the MTT assay and by the Trypan blue test, respectively, at the time indicated after LPA or serum. Data are means ± S.E.M. ($N = 3$ for C and $N = 4$ for D).

phorylation of PKC-δ after LPA treatment, suggesting that PI3K, Src and Gi proteins lay upstream of PKC-δ. Several studies suggest that Src may be the kinase responsible of PKC-δ phosphorylation [26–30], but PI3K may also be required somehow for this phosphorylation, as judged by the sensitivity to LY 294002. In this regard it has been reported that PKC-δ activation was dependent on PI3K in RIF tumor cells exposed to hypoxia [31].

3.5. Effect of LPA on the proliferation of T/C-28a2 chondrocytes

Since LPA is considered to be a mitogen for several cell types [18] and ODC induction is generally correlated to cell growth [4,5], we have investigated whether LPA was able to stimulate the proliferation of T/C-28a2 chondrocytes. As a proliferation marker, we evaluated the expression of the nuclear Ki-67 protein by Western blotting. In fact, Ki-67 is expressed in proliferating, but not in quiescent human cells, and is widely used to determine the proportion of proliferating cells in human cell populations [32]. Fig. 6A shows that Ki-67 content was reduced after 20 h of treatment with LPA, and this effect was prevented by PTX. On the contrary 10% serum, used as a positive control, provoked an increase in Ki-67 expression. DNA quantitation indicated that the number of chondrocytes was significantly reduced after 2 and 3 days of LPA treatment with respect to control (by 26% and 27%, respectively, Fig. 6B). It should be noted that the previous experiments were performed with confluent cell cultures, so growth curves were obtained starting from non-confluent cultures to evaluate more carefully the LPA effect on chondrocyte proliferation (Fig. 6C). LPA could not mimic the action of serum on cell proliferation: unlike serum, incubation of chondrocytes with LPA did not increase the number of chondrocytes, which was actually reduced with respect to control ($P < 0.05$ by paired t-test). In fact, the number of LPA-treated cells, evaluated by the MTT test, was about 20, 50 and 40% of control after 1, 2 and 3 days of treatment, respectively. This reduction in cell number was substantially confirmed by directly counting of the cells, resulting 51 ± 6.7% of control after 1 day (mean ± SEM, $N = 4$, $P < 0.05$ by unpaired t-test). Besides, the reduction in cell number was not due to a decrease in cell viability (Fig. 6D), ruling out an apoptotic or cytotoxic effect of LPA. Accordingly, the induction of effector caspase activity, critical enzymes in the executive phase of apoptosis, was not observed after LPA treatment (not shown). These results indicate that LPA is not a mitogen for human T/C-28a2 chondrocytes, but rather exerted an antiproliferative effect.

4. Discussion

LPA is a bioactive phospholipid present in serum (2–20 μM) bound to albumin [18]. Although all cells contain small amounts of LPA associated with membrane biosynthesis, LPA can be released by some cellular sources such as activated platelets, leukocytes, epithelial cells, neuronal cells and tumor cells. LPA is a potent signaling molecule able to provoke a variety of cellular responses in many cell types, including cell proliferation and differentiation [18–20]. The biological actions

of LPA are mediated by specific seven-transmembrane domain receptors, which activate various downstream signaling pathways through at least three different types of heterotrimeric G proteins [18,19]. However, the mechanisms of activation of the key proteins involved in LPA-triggered pathways and their reciprocal interactions are scarcely defined.

The present paper shows that physiological concentrations of LPA are able to activate key signaling pathways in human chondrocytes involving Gi proteins, p60 Src tyrosine kinase, PKC-δ, p44/42 MAPK and PI3K and to induce ODC markedly. Therefore, chondrocytes may be a novel target for LPA action. This study also represents the first report that LPA can induce ODC in mammalian cells. Experiments with selective inhibitors together with available data in the literature indicate that LPA leads to Src activation through specific Gi protein-coupled receptors. Src then can activate PI3K and PKC-δ. All these signaling proteins are required for ODC induction. Although fragmentary data are available about the involvement of some of these proteins in ODC expression in different cells [13,22,33,34], the possible pathway(s) and interrelations of these proteins had not been reported previously. Regarding PKC-δ, it has been shown that LPA can induce nuclear localization of PKC-δ in bovine theca cells [35]. Furthermore, PKC-δ is known to regulate gene expression through various mechanisms [36] and in particular it can be required for ODC induction in papilloma PE cells exposed to oxidative stress [33]. Interestingly, we have found a rottlerin-sensitive increase in ODC mRNA level following LPA treatment, as determined by RT-PCR (not shown).

These results raise the question of a possible function of LPA in chondrocytes. Although ODC induction is generally correlated to cell growth and polyamines are required for cell proliferation, LPA was not a mitogen for human T/C-28a2 chondrocytes. Rather it appeared to exert an anti-proliferative effect. Interestingly, some reports indicate that ODC and polyamines are good markers of differentiated chondrocytes [37,38]. Therefore, further studies will be carried out to investigate whether LPA may favour chondrocyte differentiation. Another worthwhile pursuit suggested by our findings would be to ascertain whether LPA concentration and chondrocyte response might vary in cartilage under various physiological and pathological conditions.

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