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Data Article

Early LPS-induced ERK activation in retinal pigment epithelium cells is dependent on PIP₂-PLC[☆]



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ABSTRACT

This article presents additional data regarding the study “The phospholipase D pathway mediates the inflammatory response of the retinal pigment epithelium” [1]. The new data presented here show that short exposure of RPE cells to lipopolysaccharide (LPS) induces an early and transient activation of the extracellular signal-regulated kinase (ERK1/2). This early ERK1/2 activation is dependent on phosphatidylinositol bisphosphate-phospholipase C (PIP₂-PLC). On the contrary, neither the phospholipase D 1 (PLD1) nor the PLD2 inhibition is able to modulate the early ERK1/2 activation induced by LPS in RPE cells.

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Abbreviations: ERK, extracellular signal-regulated kinase; HRP, horseradish peroxidase; LPS, lipopolysaccharide; PIP₂-PLC, phosphatidylinositol bisphosphate-phospholipase C; PLD, phospholipase D; RPE, retinal pigment epithelium

[☆]The data presented here is additional data related to the publication “The phospholipase D pathway mediates the inflammatory response of the retinal pigment epithelium” Melina V. Mateos, Constanza B. Kamerbeek, Norma M. Giusto, Gabriela A. Salvador, *Int. J. Biochem. Cell. Biol.*, vol. 55, October 2014, pp. 119–128, doi: 10.1016/j.biocel.2014.08.016, Epub 2014 August 27.

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Specifications table

Subject area	<i>Biochemistry</i>
More specific subject area	<i>Cell biology</i>
Type of data	<i>WB images, bar graphs</i>
How data was acquired	<i>Western blot. Densitometry values were obtained using the ImageJ software</i>
Data format	<i>Raw and analyzed</i>
Experimental factors	<i>ARPE-19 cells were exposed to LPS. Pharmacological inhibitors of PLD1, PLD2 and PIP₂-PLC were used.</i>
Experimental features	<i>ERK1/2 activation was evaluated by Western blot</i>
Data source location	<i>Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB), Universidad Nacional del Sur (UNS) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), 8000 Bahía Blanca, Argentina.</i>
Data accessibility	<i>Data is provided within the article</i>

Value of the data

- The data can be useful to other scientists investigating the effects of LPS on RPE cells.
- The data provide additional information regarding the LPS-induced ERK1/2 signaling in RPE cells.
- Results shown here demonstrate that the early and the late LPS-induced ERK1/2 activation are differentially modulated by PIP₂-PLC and PLD pathways.

1. Data

The data presented here show that in RPE cells, ERK1/2 activation induced by 5 min treatment with LPS depends on PIP₂-PLC but is not affected by classical PLDs inhibition.

2. Experimental design, materials and methods

2.1. Retinal-pigmented epithelium cell culture and treatments

Human retinal-pigmented epithelium cells (ARPE-19) were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS, Natocor, Argentina), 100 U/ml penicillin, 100 µg/ml streptomycin and 0.25 µg/ml amphotericin B at 37 °C under 5% CO₂. Confluent 35 mm diameter cell dishes were serum-starved for 2 h prior to stimulation for 5 min or 2 h with 10 µg/ml of *Pseudomonas aeruginosa* LPS in serum-free DMEM. Sterile ultra pure water was added to the control condition. In order to inhibit PIP₂-PLC and PLDs pathways ARPE-19 cells were preincubated with selective inhibitors for 1 h at 37 °C prior to cell stimulation with LPS. 0.15 µM EVJ was used to inhibit PLD1 activity and 0.5 µM APV to inhibit PLD2. For PIP₂-PLC inhibition, cells were preincubated with U73122 (10 µM). DMSO (vehicle of the inhibitors) was added to all conditions to achieve a final concentration of 0.025% [1,2].

2.2. Western blot analysis (WBs)

After experimental treatment the medium was removed, cells were washed three times with PBS and scraped off with 80 μ l ice-cold RIPA lyses buffer (10 mM Tris-HCl (pH 7.4), 15 mM NaCl, 1% Triton X-100, 5 mM NaF, 1 mM Na_2VO_4 and the complete protease inhibitor cocktail). Protein content of cellular lysates was determined by Bradford method [3] (Bio-Rad Life Science group, 500-0006). Samples were denatured with Laemmli sample buffer at 100 °C for 5 min [4]. Equivalent amounts of proteins (30 μ g) were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on 10% polyacrylamide gels, transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, Bedford, MA, USA) and WBs were performed as previously described [1]. Rabbit polyclonal antibody anti-phospho-ERK1/2 (9101) was from Cell Signaling (Beverly, MA, USA). Mouse monoclonal anti- α Tubulin (DM1-A) (CP06) was from EMD/Biosciences-Calbiochem (San Diego, CA, USA). HRP-conjugated goat anti-rabbit IgG and HRP-conjugated goat anti-mouse IgG were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Densitometry values of the immunoreactive bands were determined using ImageJ 1.38 software.

2.3. Statistical analysis

Statistical analysis was performed using ANOVA followed by Bonferroni's test to compare means. *p*-Values lower than 0.05 were considered statistically significant. Data represent the mean value \pm SD of at least three independent experiments. The WBs shown are a representative image of samples from at least three independent experiments.

3. Results

As shown in Fig. 1, 5 min exposure to LPS (10 μ g/ml) induced a strong activation of ERK1/2 (120% compared to the control condition) in ARPE-19 cells. This activation is transient since it is no longer

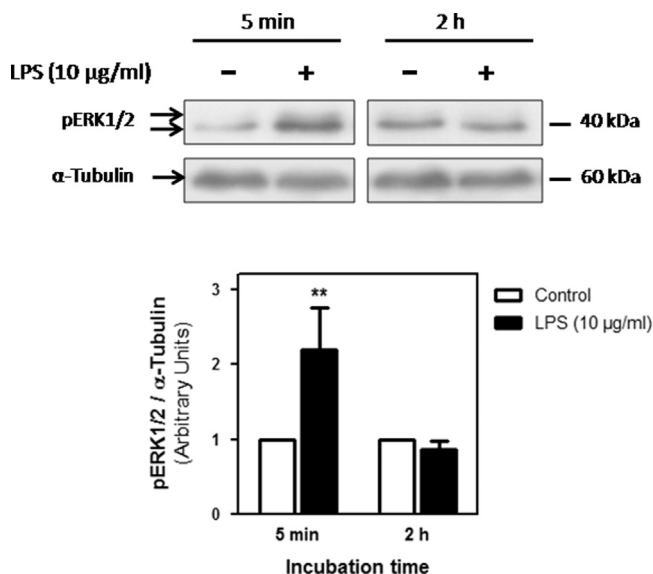


Fig. 1. ERK1/2 activation in ARPE-19 cells exposed to LPS. ARPE-19 cells were treated with LPS (10 μ g/ml) or ultra pure water (control condition) for 5 min or 2 h. ERK1/2 activation was evaluated by WB assays using anti-phospho ERK1/2 (pERK1/2) antibody. Numbers to the right indicate molecular weights and the bar graph shows the densitometry values of pERK1/2/ α -Tubulin expressed as arbitrary units as ratio of the control. Asterisks indicate significant differences with respect to the control condition (***p* < 0.01).

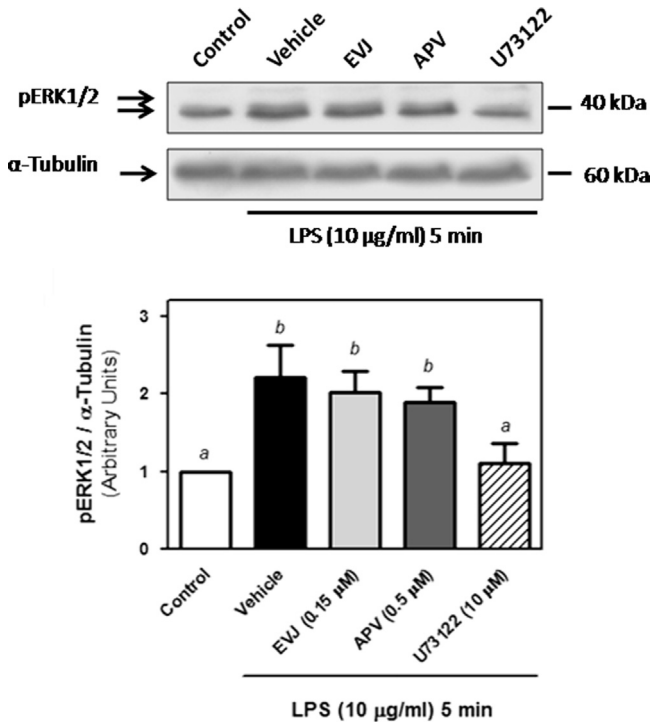


Fig. 2. Role of PIP₂-PLC and PLDs in ERK1/2 activation. ARPE-19 cells were preincubated with 0.025% DMSO (control and vehicle conditions), 0.15 μ M EVJ, 0.5 μ M APV or 10 μ M U73122 for 1 h before LPS treatment. Cells were treated with 10 μ g/ml LPS or ultra pure water (control condition) for 5 min. Numbers to the right indicate molecular weights and the bar graph shows the densitometry values of pERK1/2/ α -Tubulin expressed as arbitrary units as ratio of the control. (a and b) Indicate significant differences among columns ($p < 0.05$).

observed after 2 h treatment with LPS. The transient ERK1/2 activation observed after 5 min stimulation with LPS was only prevented by the PIP₂-PLC inhibitor U73122 but was not affected by PLD1 or PLD2 inhibition (Fig. 2). These data demonstrate that the LPS-induced early ERK1/2 depends exclusively on PIP₂-PLC (Fig. 2), while PLD2 activation is necessary to maintain ERK1/2 phosphorylation after 24 h treatment [1].

Acknowledgments

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.dib.2016.02.057>.

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