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Involvement of Oxidative Pathways in Cytokine-induced Secretory Phospholipase A2-IIA in Astrocytes

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Abstract

Recent studies have suggested the involvement of secretory phospholipase A2-IIA (sPLA2-IIA) in neuroinflammatory diseases. Although sPLA2-IIA is transcriptionally induced through the NF-κB pathway by pro-inflammatory cytokines, whether this induction pathway is affected by other intracellular signaling pathways has not been investigated in detail. In this study, we demonstrated the induction of sPLA2-IIA mRNA and protein expression in astrocytes by cytokines and detected the protein in the culture medium after stimulation. We further investigated the effects of oxidative pathways and botanical antioxidants on the induction pathway and observed that IL-1β-induced sPLA2-IIA mRNA expression in astrocytes is dependent on ERK1/2 and PI-3 kinase, but not p38 MAPK. In addition to apocynin, a known NADPH oxidase inhibitor, botanical antioxidants, such as resveratrol and epigallocatechin gallate, also inhibited IL-1β-induced sPLA2-IIA mRNA expression. These compounds also suppressed IL-1β-induced ERK1/2 activation and translocation of the NADPH oxidase subunit p67 phox from cytosol to membrane fraction. Taken together, these results support the involvement of reactive oxygen species from NADPH oxidase in cytokine induction of sPLA2-IIA in astrocytes and promote the use of botanical antioxidants as protective agents for inhibition of inflammatory responses in these cells.

Keywords

secretory phospholipase A2-IIA; astrocytes; NADPH oxidase; MAP kinases; apocynin; resveratrol; epigallocatechin gallate (EGCG)

1. INTRODUCTION

Phospholipases A2 (PLA2, EC3.1.1.4.) catalyze the hydrolysis of *sn*-2 fatty acids from phospholipids. There are more than 20 distinct mammalian isoforms of PLA2 belonging to the calcium-dependent cytosolic group IV PLA2 (cPLA2), the calcium-independent group VI PLA2 (iPLA2), or the small molecular weight group II secretory PLA2 (sPLA2) (Murakami

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et al., 1997; Sun et al., 2004; Sun et al., 2007; Burke and Dennis, 2008a, b). These enzymes are widely expressed across mammalian cell types and besides playing a role in maintaining integrity of phospholipids in the cell membrane, they are also involved in the production of arachidonic acid, a precursor for prostanoids.

Among more than 12 isoforms of sPLA2, considerable attention has been given to the sPLA2-IIA and inhibitors for this type of PLA2 (Boilard et al., 2006; Lambeau and Gelb, 2008; Oslund et al., 2008; Ibeas et al., 2009). This enzyme is a mediator connecting innate and adaptive immunity and is up-regulated in a number of coronary artery diseases, including atherosclerosis, sepsis, arthritis and infection (Leitinger et al., 1999; Tietge et al., 2005; Krijnen et al., 2006; Mallat et al., 2007; Kimura-Matsumoto et al., 2008; Ibeas et al., 2009). Up-regulation of sPLA2-IIA mRNA expression and immunoreactivity has been reported in rat brain after cerebral ischemia (Lin et al., 2004; Adibhatla and Hatcher, 2007) and in human Alzheimer's disease brain (Moses et al., 2006). Furthermore, in vitro studies demonstrated the critical role of sPLA2-IIA in neuronal channels and activity (Kolko et al., 2002; Yagami et al., 2002; Mathisen et al., 2007).

Our earlier studies with cultured astrocytes provided evidence for the induction of sPLA2-IIA by inflammatory cytokines, such as interleukin 1-beta (IL-1β) and tumor necrosis factor alpha (TNFα) (Sun and Hu, 1995; Xu et al., 2003a). Subsequent studies indicated that sPLA2-IIA is transcriptionally induced by pro-inflammatory cytokines through the NF-κB pathway (Sun and Hu, 1995; Tong et al., 1999; Lappas et al., 2004; Jaulmes et al., 2005). Nevertheless, whether transcriptional synthesis of sPLA2-IIA is regulated by other signaling cascades has not been explored in sufficient detail.

Recent studies provided evidence that a phagocyte-like NADPH oxidase, capable of generating reactive oxygen species (ROS) in the form of superoxide, is functionally active in astrocytes (Noh and Koh, 2000; Abramov et al., 2005; Liu et al., 2005). This NADPH oxidase contains both membrane and cytoplasmic components, and its activation has been linked to a number of cell surface receptors and signaling cascades (Bedard and Krause, 2007). In this study, we examined the involvement of the NADPH oxidase and other oxidative signaling pathways in cytokine induction of sPLA2-IIA in astrocytes. In addition, we tested the effects of the botanical antioxidants resveratrol and epigallocatechin gallate (EGCG) on sPLA2-IIA expression.

2. MATERIALS AND METHODS

2.1. Materials

Apocynin, anti-β-actin antibody and BSA were purchased from Sigma (St. Louis, MO, USA). Cytokines were purchased from R & D Systems (Minneapolis, MN, USA). SB203580 and U0126 and LY294002 were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Trizol, and Superscript III One Step RT-PCR kit were purchased from Invitrogen (Eugene, OR, USA). Oligonucleotide primers were obtained from Integrated DNA Technologies (Coralville, IA, USA). DITNC (immortalized rat astrocytes) were obtained from ATCC (Rockville, MD, USA). Dulbecco's modified Eagle's medium (DMEM), DMEM:F12 1:1, and TryPLE (trypsin) were purchased from GIBCO-BRL (Gaithersburg, MD, USA). Polyclonal antibodies for sPLA2-IIA were obtained from BioVendor (Candler, NC, USA). Anti-phospho-ERK1/2 and anti-ERK1/2 were obtained from Cell Signaling Technology (Danvers, MA, USA), and p67 phox antibodies were obtained from Upstate (Billerica, MA, USA). SuperSignal West Pico chemiluminescence was purchased from Pierce (Rockford, IL, USA).

2.2. Cell culture

The immortalized rat astrocyte cell line (DITNC) was maintained in DMEM with 10% FBS and 1% penicillin/streptomycin (P/S) at 37 °C with 5% CO2 and 95% humidity. Prior to experiments, cells were starved for 4 hours in DMEM medium, followed by treatments with different conditions as described. Polyphenols and inhibitors were added to the cell medium 1 h before treatment with cytokines for 18 hours.

2.3. Crude membrane preparation

In experiments requiring preparation of crude membrane fractions, astrocytes were pretreated with resveratrol or EGCG followed by exposing to IL-1β for 10 min. Cells were then suspended in 0.25 M sucrose, 5 mM $MgCl_2$, 2 mM EGTA, 2 mM EDTA, 10 mM Tris (pH 7.5), 10 μg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 μg/ml pepstatin, and 10 μg/ml aprotinin and were disrupted by brief sonication and centrifuged at 1000×*g* for 5 min at 4 °C to remove unbroken cells and nuclei. Supernatant was removed and centrifuged at 100,000×*g* for 1 h at 4 °C (SW40 rotor, Beckman ultracentrifuge). The membrane pellet was resuspended in buffer with 0.5% (v/v) Triton X-100 for 1 h at 4 °C (Min *et al.* 2004) and expression of p67 phox protein was analyzed by Western blot.

2.4. Western blot analysis

Astrocytes were cultured in 60 mm or 100 mm dishes until 90% confluent. After treatment, cells were washed with ice-cold PBS twice, followed by lysing with lysis buffer (50 mM Tris-HCl, pH 7.4, 1 mM EDTA, 100 mM NaCl, 0.1% SDS, 1 mM PMSF, 1 mM sodium orthovanadate, 1 μg/ml leupeptin, 1 μg/ml pepstatin, 10 μg/ml aprotinin). Protein concentrations were determined by the Bradford assay (Bradford, 1976). Equivalent amounts of protein for each sample were resolved in a 15% SDS-Page and run for 60 minutes at 200 V. Proteins were subsequently transferred at 100 V for 1 h to nitrocellulose membranes. Membranes were incubated in Tris-buffered saline, pH 7.4, with 0.5% Tween 20 (TBS-T) containing 5% nonfat milk for 1 h at room temperature. Blots were reacted with the primary antibody (TBS-T with 5% milk) at 4 °C overnight, washed 3 times for 5 min in TBS-T, and then incubation with the secondary antibody (TBS-T with 5% milk) for 1 h at room temperature. After washing 3 times for 5 min with TBS-T, SuperSignal West Pico chemiluminescence reagents from Pierce were used to signal detection. Band density was measured using Quantity One software (Bio-Rad, Hercules, CA, USA). In some studies, the blots were striped using the standard protocol, washed and re-probed with anti-β-actin. The following antibody concentrations or dilutions were used: sPLA2-IIA (1: 1000), phospho-ERK1/2 (1:1000), ERK1/2 (1:2000), p67 phox (1:1000), β-actin (1:30000) secondary antibodies (1:5000).

2.5. Semi-quantitative RT-PCR

Cells were washed with PBS following treatment and RNA was isolated with Trizol (Invitrogen) according to manufacturer's instructions. A SuperScript III One-Step kit (Invitrogen) was used according to manufacturer's instructions. Briefly, one microgram of RNA was used in the one-step RT-PCR with 50 pmol of the following oligonucleotide primers designed from rat gene sequences for sPLA2-IIA: sense 5′-TGACTCATGACTGTTGTTACAACC-3′ and antisense 5′-TCTCAGGACTCTCTTAGGTACTA-3′ (amplifies a 493 bp fragment); and for β-actin: sense 5′-TGGAGAAGAGCTATGAGCTGCCTG-3′ and antisense 5′-GTGCCACCAGACAGCACTGTGTTG-3′ (amplifies a 289 bp fragment). Reverse transcription at 50°C for 30 min and inactivation of reverse transcriptase at 94 °C for 2 min were followed by 40 cycles of amplification for sPLA2-IIA cDNA (annealing at 55 °C for 1 min) or 30 cycles of amplification for β-actin cDNA (annealing at 55°C for 1 min). Each cycle included a 15 s denaturation step at 94 °C, an annealing step, and a 1 min extension step at 68

°C. A 5 min extension at 68 °C was carried out at the end of the final cycle. Amplified DNA was visualized on a 2% agarose gel with ethidium bromide and analyzed with Quantity One software.

2.6. Reverse transcription

Reverse transcription of RNA was carried out using the Taqman Reverse Transcription Kit following the manufacturer's instructions (Ambion, Austin, TX, USA). Briefly, RNA concentration was determined with spectrophotometry at 260 nm and concentrations of samples were normalized to 30 μg/ml. Oligo $d(T)_{16}$ was combined with other reaction components, and 6.15 μl of reaction mix was added to each tube containing 3.85 μl of RNA for a total volume of 10 μl. The samples were incubated at 25 °C for 10 min, followed by the reverse transcription step at 48 °C for 30 min, and reverse transcriptase inactivation at 95 °C for 5 min.

2.7. Quantitative real-time PCR

The ABI 7300 Taqman by Applied Biosystems was used for real-time PCR according to manufacturer's instructions. A pre-designed primer and probe set was purchased for determination of expression of sPLA2-IIA (Assay ID Rn00580999) based on NCBI sequence NM 031598.1 with an amplicon length of 94 bp. A β-actin primer and probe was used as a control, with the following sequences: Forward 5′GCCCTGGCTCCTAGCACC-3′, Reverse 5′CCACCAATCCACACAGAGTACTTG-3′, and Probe 5′ TGAAGATCAAGTCATTGCTCCTCCTGAGC-3′ with a FAM modification on the 5′ end, having an amplicon length of 73 bp. Briefly, 5 μl cDNA was combined with Taqman Universal PCR Master Mix, along with a FAM-MGB probe and primers, for a final concentration of 25 μl. The reaction plate was incubated as follows: 50 °C for 2 min, 95 °C for 10 min, and 50 cycles of denature (95 °C for 15 sec) and anneal/extend (60 °C for 1 min). The C_T was determined and sPLA2-IIA transcript concentration was normalized against that of β-actin.

2.8. Statistical analysis

Data were analyzed by one-way ANOVA followed by Newman-Keuls multiple comparison test (V4.00, GraphPad Prism Software, Inc., San Diego, CA). Values of $p<0.05$ were accepted as significant.

3. RESULTS

3.1. Cytokine induction of sPLA2-IIA mRNA and protein expression in rat immortalized astrocytes (DITNC)

In the initial study, immortalized astrocytes (DITNC) were tested for induction of sPLA2-IIA mRNA expression by pro-inflammatory cytokines. The RT-PCR study indicated induction of sPLA2-IIA mRNA upon exposure to IL-1β and TNFα for 18 h (Fig 1A). Exposure of astrocytes with interferon gamma (IFNγ) alone did not cause the induction of sPLA2-IIA mRNA (data not shown).

Western blot analysis was used to estimate sPLA2-IIA concentrations in the cell lysates and in the culture medium. As shown in Fig 1B, exposure of astrocytes to TNFα and IL-1β for 48 h induced visible sPLA2-IIA protein in both cell lysate and in the culture medium. Similar induction profile was observed in primary rat astrocytes (data not shown).

3.2. Involvement of protein kinases in the induction of sPLA2-IIA mRNA and protein by IL-1β

We tested the effects of several protein kinases, i.e., ERK1/2, p38 MAPK and PI-3 kinase, on induction of sPLA2-IIA by IL-1β. The MEK inhibitor, U0126, which inhibits MEK1/2, a direct activator of ERK1/2, was effective in inhibiting IL-1β-induced expression of sPLA2-IIA mRNA and protein (Fig 2). However, SB203580, an inhibitor for p38 MAPK, did not alter IL-1β induced sPLA2-IIA expression (data not shown). As shown in Figure 3, IL-1β induced sPLA2-IIA upregulation in DITNC cells was significantly attenuated by LY294002, a known inhibitor of PI-3 kinase.

3.3. Inhibition of IL-1β-induced sPLA2-IIA mRNA expression by apocynin

We tested the possible involvement of NADPH oxidase in mediating IL-1β-induced sPLA2-IIA expression by treating astrocytes with apocynin, an inhibitor known to block the translocation of cytoplasmic subunits from docking with membrane subunits of NADPH oxidase (Stolk et al., 1994). As shown in Fig 4, apocynin at 500 μM effectively inhibited IL-1β-induced PLA2-IIA mRNA expression in astrocytes. Apocynin as well as the kinase inhibitors showed little to no effect on cell viability as demonstrated by MTT test (data not shown).

3.4. Botanical polyphenols inhibited IL-1β-induced sPLA2-IIA mRNA through NADPH oxidase pathway

In this experiment, we tested the effects of resveratrol and EGCG on IL-1β-induced sPLA2-IIA in astrocytes. Our results show that pretreatment of astrocytes with resveratrol resulted in an inhibition of IL-1β-induced expression of sPLA2-IIA mRNA (Fig 5). Similarly, pretreatment of astrocytes with EGCG also resulted in a dose-dependent inhibition of IL-1β-induced sPLA2-IIA mRNA expression (Fig 6).

With both resveratrol and EGCG inhibiting sPLA2-IIA mRNA expression, we further tested whether these compounds inhibit the translocation of the soluble NADPH oxidase subunit p67 phox to membranes. After pretreatment with resveratrol or EGCG, astrocytes were exposed to IL-1β for 10 min and expression of p67 phox protein in the membrane fraction was examined by Western blot analysis. As shown in Fig 7, IL-1β induced the translocation of p67 phox to the membrane fraction and resveratrol and EGCG each inhibited this translocation.

3.5. Apocynin and botanical polyphenols inhibited IL-1β-induced ERK1/2 phosphorylation

Since ERK1/2 has been shown to be activated following NADPH oxidase activation in astrocytes (Pawate et al., 2004), we investigated the effects of apocynin, resveratrol, and EGCG on the phosphorylation of ERK1/2 after stimulation of astrocytes with IL-1β. As shown in Fig 8, all three compounds inhibited IL-1β-induced increase in phospho-p42 ERK.

4. DISCUSSION

The importance of sPLA2-IIA in neurodegenerative diseases, especially in association with inflammatory processes has started to emerge (Adibhatla and Hatcher, 2007; Sun et al., 2007). Studies linking this enzyme with transgenic mouse models have been hampered due to a missense mutation in the sPLA2-IIA gene in a number of mouse strains (Kennedy et al., 1995). However, studies with rat brain demonstrated the inflammatory properties of this enzyme and its upregulation in astrocytes by pro-inflammatory cytokines including IL-1β and TNFα (Oka and Arita, 1991; Tong et al., 1999; Rosenberger et al., 2004; Moses et al., 2006). In the present study, our Western blot analysis demonstrates for the first time that cytokine-induced sPLA2-IIA protein is secreted into the culture medium. This result is in agreement

with our previous study showing active PLA2 activity in the culture media using radioactive phospholipids as substrate (Xu et al., 2003).

Although a number of studies have demonstrated the transcriptional induction of sPLA2-IIA through the NF-κB pathway (Andreani et al., 2000; Lappas et al., 2004), less is known about the roles of other signaling molecules in mediating the induction. In this study, we used real-time PCR to measure sPLA2-IIA mRNA expression in astrocytes, and demonstrated the involvement of ERK1/2 and PI-3 kinase (but not p38 MAPK) in sPLA2-IIA expression by IL-1β. In the CNS, ERK1/2 are important signaling molecules that integrate extracellular signals (Sweatt, 2001; Chu et al., 2004). Furthermore, ERK1/2 activation is implicated in a large number of intracellular factors, including Ca^{2+}, PKC, nitric oxide, and PI-3 kinase (Andersen et al., 2003). In our recent studies with cortical neurons, ERK1/2 phosphorylation was stimulated by ROS produced by NADPH oxidase and in turn this signaling pathway led to activation of cPLA2 (Shelat et al., 2008). Other studies have also demonstrated that NADPH oxidase-generated ROS can activate the ERK1/2 pathway and vice versa, ERK1/2 activation can stimulate the phosphorylation of cytosolic subunits of NAPDH oxidase leading to an increased activity of the enzyme (Pawate et al., 2004; Miller et al., 2007; Yang et al., 2007a). In microglial cells, LPS-induced activation of PI-3 kinase and p38 MAPK (but not ERK1/2) pathways is dependent on ROS production by NADPH oxidase (Sun et al., 2008). These kinases are important in glial redox signaling and their inhibition can lead to the reduced production of inflammatory proteins (Bhat et al., 1998; Saha and Pahan, 2006; Yang et al., 2007b). Our results are in agreement with these findings. Interestingly, PKC activation of PI-3 kinase (Frey et al., 2006) can modulate sPLA2-IIA expression in IL-1β treated mesangial cells (Scholz et al., 1999). Apparently, depending on the cell types, cytokines may stimulate different kinase pathways and regulate the transcriptional events leading to sPLA2-IIA expression.

Although mechanism not well understood, ROS have been shown to play a role as second messengers in NF-κB activation (Schreck et al., 1992; Baeuerle and Henkel, 1994; Flohe et al., 1997). In this study, we provided evidence that NADPH oxidase-derived ROS is necessary for IL-1β induction of sPLA2-IIA. Our results are in accordance with other findings which demonstrated the involvement of NADPH oxidase in neuroinflammatory processes, especially glial activation (Brown, 2007). NADPH oxidase is considered an important non-mitochondrial source of oxidative stress in the brain and has been implicated in a number of neurodegenerative diseases including Alzheimer's, Parkinson's, HIV dementia, ischemic stroke, and multiple sclerosis (Bedard and Krause, 2007). The involvement of NADPH oxidase in IL-1β-mediated induction of sPLA2-IIA was demonstrated by inhibition with apocynin, a phenolic compound extracted from *Picrorhiza kurroa*, a creeping plant native to the mountains of India, Nepal, Tibet and Pakistan (Wang et al., 2008). Apocynin has been shown to block the activity of NADPH oxidase by interfering with the assembly of the cytosolic subunits with the membrane components (Stolk et al., 1994). The ability for apocynin to inhibit translocation of p67 phox to membranes is also indication for the involvement of NADPH oxidase in this process.

Due to their antioxidant properties, there is considerable interest to examine whether polyphenolic compounds including resveratrol from grapes and EGCG from green tea may offer protective effects and ameliorate progression of neurodegenerative diseases (Joseph *et al.* 2000, Son *et al.* 2008, Zaveri 2006, Mancuso *et al.* 2007, Cucciolla *et al.* 2007). Our earlier studies have demonstrated the abilities of resveratrol and also apocynin to protect against neuronal injury and glial activation due to cerebral ischemia-reperfusion (Wang et al., 2002; Wang et al., 2006). Other studies also demonstrated the ability of antioxidant botanical compounds to suppress the NF-κB pathway (Packer, 1998; Lavrovsky et al., 2000; Cindrova-Davies et al., 2007). Indeed, recent findings have indicated that the neuroprotective actions of these compounds arise from their modulation of important cellular signaling pathways (Mandel et al., 2004). In the current study, apocynin, resveratrol and EGCG were shown to inhibit

IL-1β-induced sPLA2-IIA mRNA expression in astrocytes. The fact that these compounds could inhibit p67 phox translocation and IL-1β induced ERK activation further supports the notion that these antioxidants may act by targeting the NADPH oxidase pathway. Our results are in agreement with recent study indicating that both resveratrol and EGCG could inhibit NADPH oxidase activity in endothelial cells (Steffen et al., 2008).

In conclusion, this study provides evidence that protein kinases and oxidative pathways, most likely the NADPH oxidase pathway, promote the expression of sPLA2-IIA mRNA in astrocytes. This study further provides support for the down regulation of this inflammatory protein by inhibitors of NADPH oxidase and botanical antioxidants.

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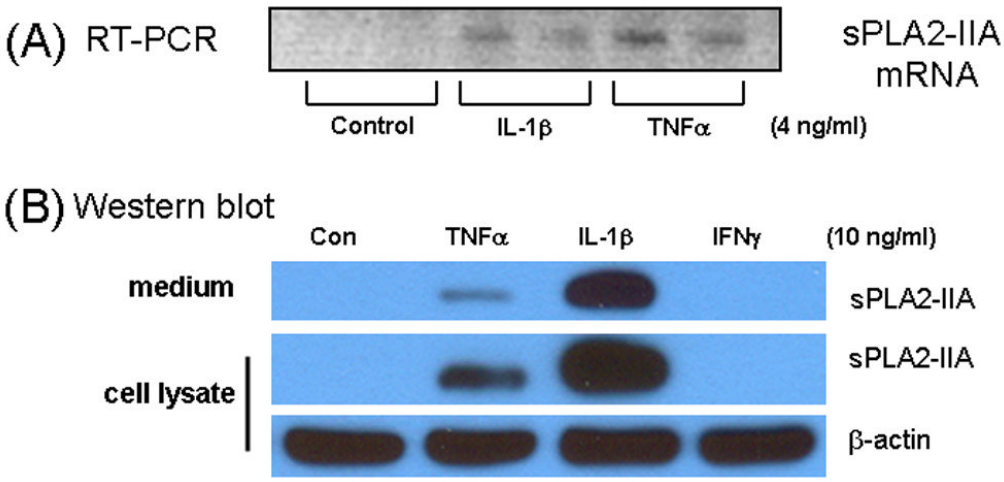

Fig 1.

(A) Induction of sPLA2-IIA mRNA in immortalized rat astrocytes (DITNC) by IL-1β and TNFα. Astrocytes were treated with 4 ng/ml with IL-1β or TNFα for 18 h prior to measurement of sPLA2-IIA mRNA expression by RT-PCR. Results are representative of three independent experiments. (B) Induction of sPLA2-IIA protein in astrocytes. DITNC astrocytes were cultured in 60 mm dish and serum-starved for 4 h prior to treatment with TNFα, IL-1β and IFNγ (10 ng/ml) for 48 h. After treatment, culture medium was removed, cells washed with PBS and lysis buffer was added. Medium (40 μl) and cell lysate (15 μg protein) was used in Western blot as described in text. β-actin in cell lysate was used as control.

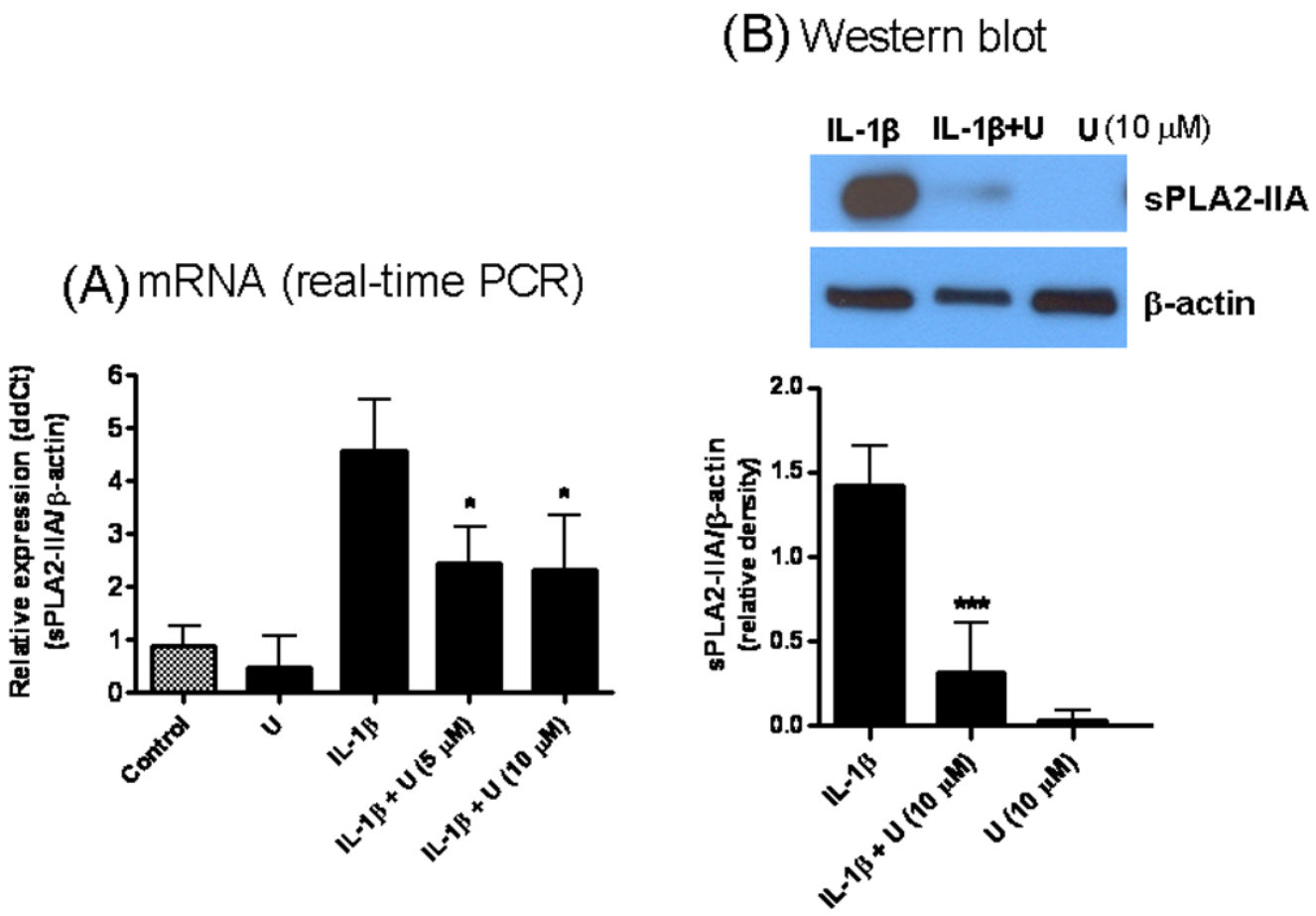

Fig 2.

U0126 (U), a MEK inhibitor, inhibited IL-1β-induced sPLA2-IIA mRNA and protein expressions in astrocytes. (A) For mRNA expression, astrocytes were treated with U0126 (10 μM) alone, IL-1β (4 ng/ml) alone and IL-1β with U0126 (5 and 10 μM) for 18 h. U0126 was added to astrocytes 1 h before treating with IL-1β. sPLA2-IIA expression was measured by real-time PCR relative to β-actin expression as described in text. Results are mean ± SD from three experiments. (B) For protein expression, astrocytes were treated with U0126 (10 μM), IL-1β (10 ng/ml), and IL-1β + U0126 for 48 h. U0126 was added 1 h prior to adding IL-1β. After incubation, cell lysates were taken for Western blot analysis as described in text. β-actin was used as control. Results are mean ± SD from three experiments. *$p<0.05$, **$p<0.01$, ***$p<0.001$ vs. IL-1β.

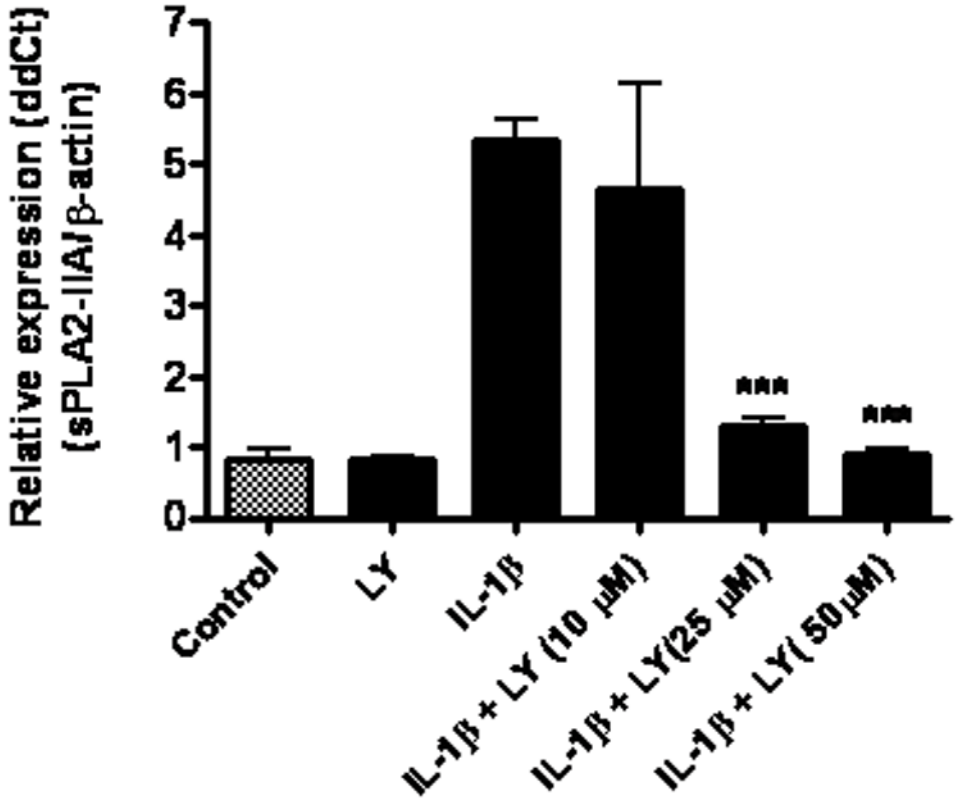

Fig 3.

LY294002, a PI-3 kinase inhibitor, inhibited IL-1β-induced sPLA2-IIA mRNA expression in astrocytes. Astrocytes were treated with LY294002 (LY, 50 μM), IL-1β (4 ng/ml), and IL-1β with LY at 10, 25, and 50 μM for 18 h. LY was added to astrocytes 1 h prior to treatment with IL-1β. sPLA2-IIA mRNA expression was determined by real-time PCR with β-actin as control. Results are mean ± SD from three experiments. ***$p<0.001$ vs. IL-1β.

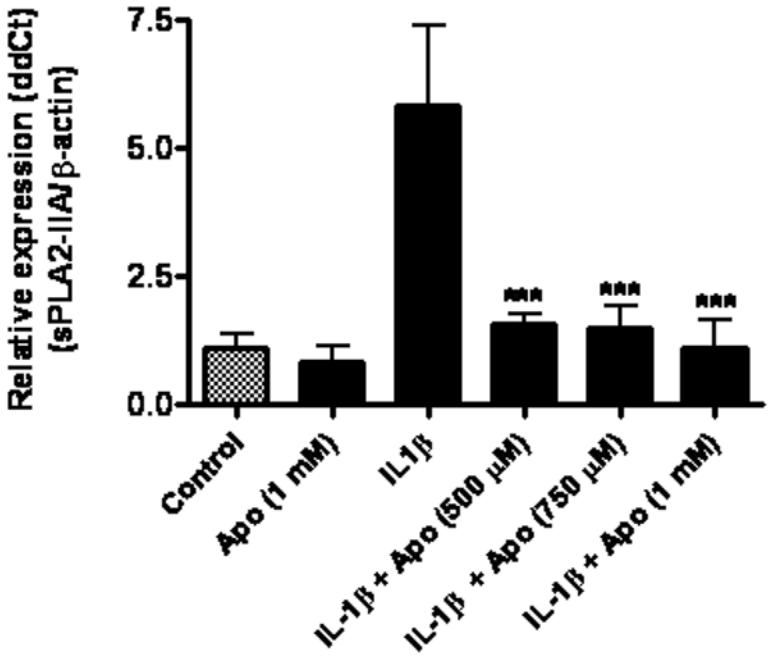

Fig 4.

Apocynin, an NADPH oxidase inhibitor, inhibited IL-1β-induced sPLA2-IIA mRNA expression in astrocytes. Astrocytes were treated with apocynin (Apo, 1 mM), IL-1β (4 ng/ml), and IL-1β with apocynin at 500, 750 and 1000 μM for 18 h. Apocynin was added to astrocytes 1 h prior to treatment with IL-1β. sPLA2-IIA mRNA expression was determined using real-time PCR with β-actin as control. Results are mean ± SD from three experiments. ***$p<0.001$ vs. IL-1β.

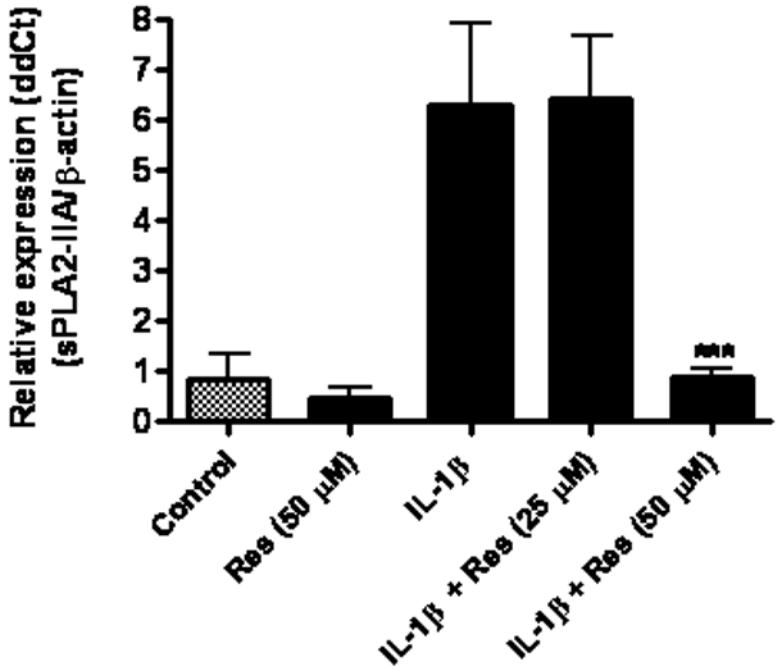

Fig 5.

Resveratrol (Res) inhibited IL-1β-induced sPLA2-IIA mRNA expression in astrocytes. Astrocytes were treated with resveratrol (50 μM), IL-1β (4 ng/ml) and IL-1β with resveratrol at 25 or 50 μM for 18 h. Resveratrol was added to astrocytes 1 h prior to treatment with IL-1β. sPLA2-IIA mRNA expression was determined using real-time PCR with β-actin as control. Results are mean ± SD from three experiments. ***$p<0.001$ vs. IL-1β.

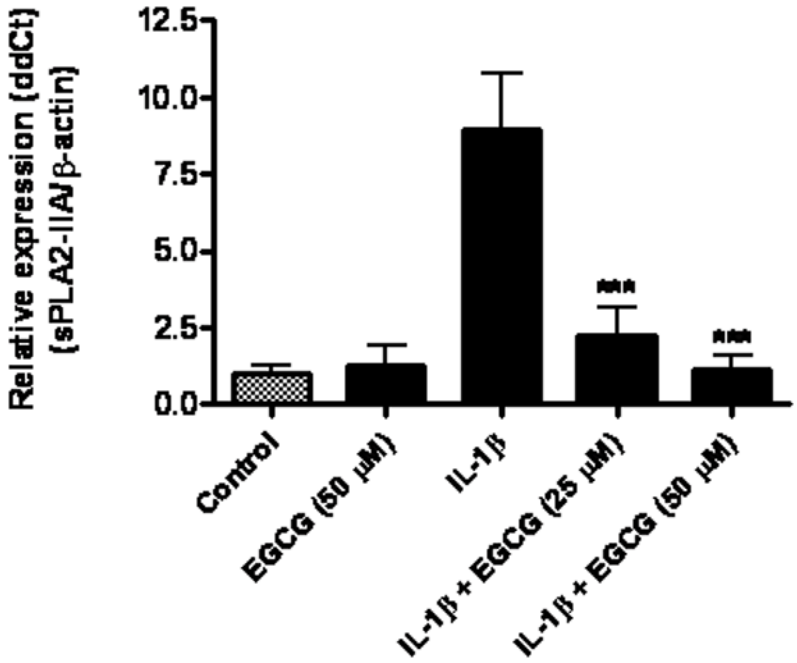

Fig 6.

Epigallocatechin gallate (EGCG) inhibited IL-1β-induced sPLA2-IIA mRNA expression in astrocytes. Astrocytes were treated with EGCG (50 μM), IL-1β (4 ng/ml) and IL-1β with EGCG at 25 or 50 μM for 18 h. EGCG was added to astrocytes 1 h prior to treatment with IL-1β. sPLA2-IIA mRNA expression was determined using real-time PCR with β-actin as control. Results are mean ± SD from three experiments. ***$p<0.001$ vs. IL-1β.

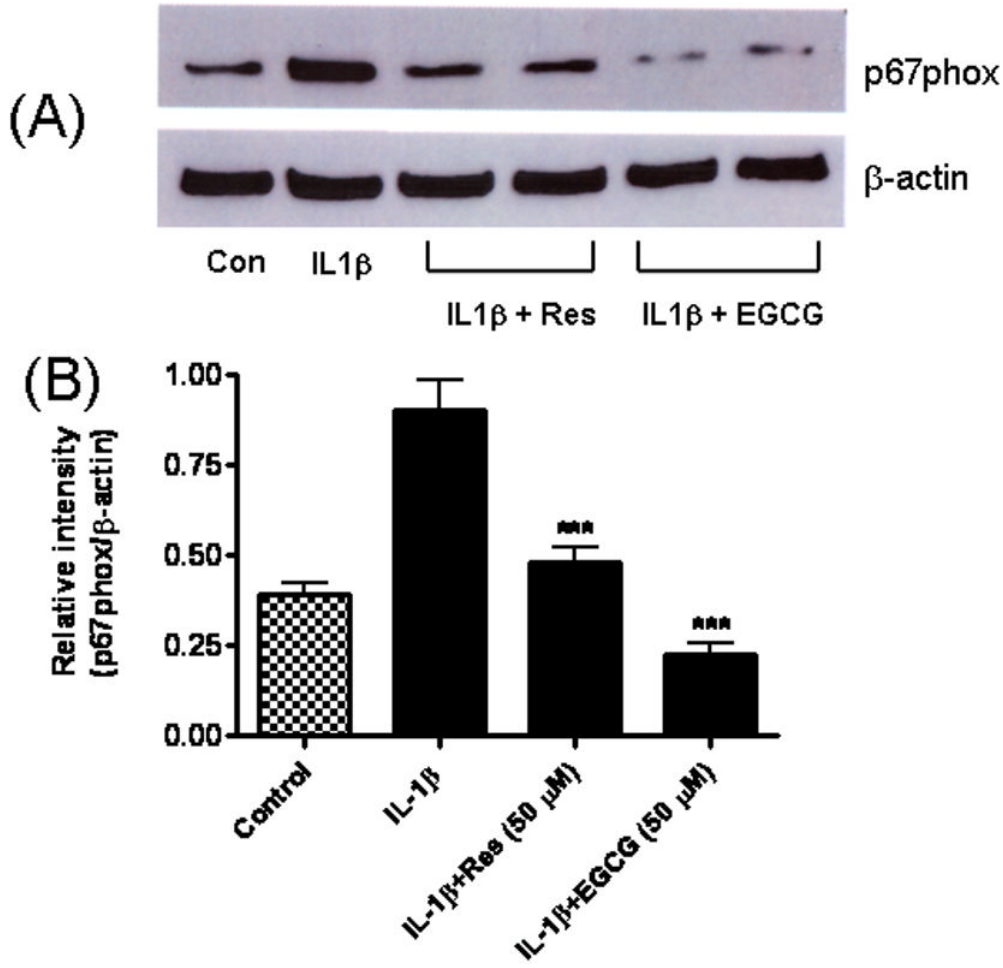

Fig 7.

Resveratrol and EGCG inhibited IL-1β-induced translocation of p67 phox to the membrane fraction. Astrocytes were treated with IL-1β (4 ng/ml) or IL-1β plus Res (50 μM) and EGCG (50 μM) for 10 min. Cells were disrupted and cell cytosol and membrane fractions were separated by centrifugation as described in Method section. (A) Western blot analysis of p67 phox concentration in the membrane fraction compared to β-actin as control. (B) Relative intensity of p67 phox compared to β-actin. Results are mean ± SD from three independent experiments. ***$p<0.001$ vs. IL-1β.

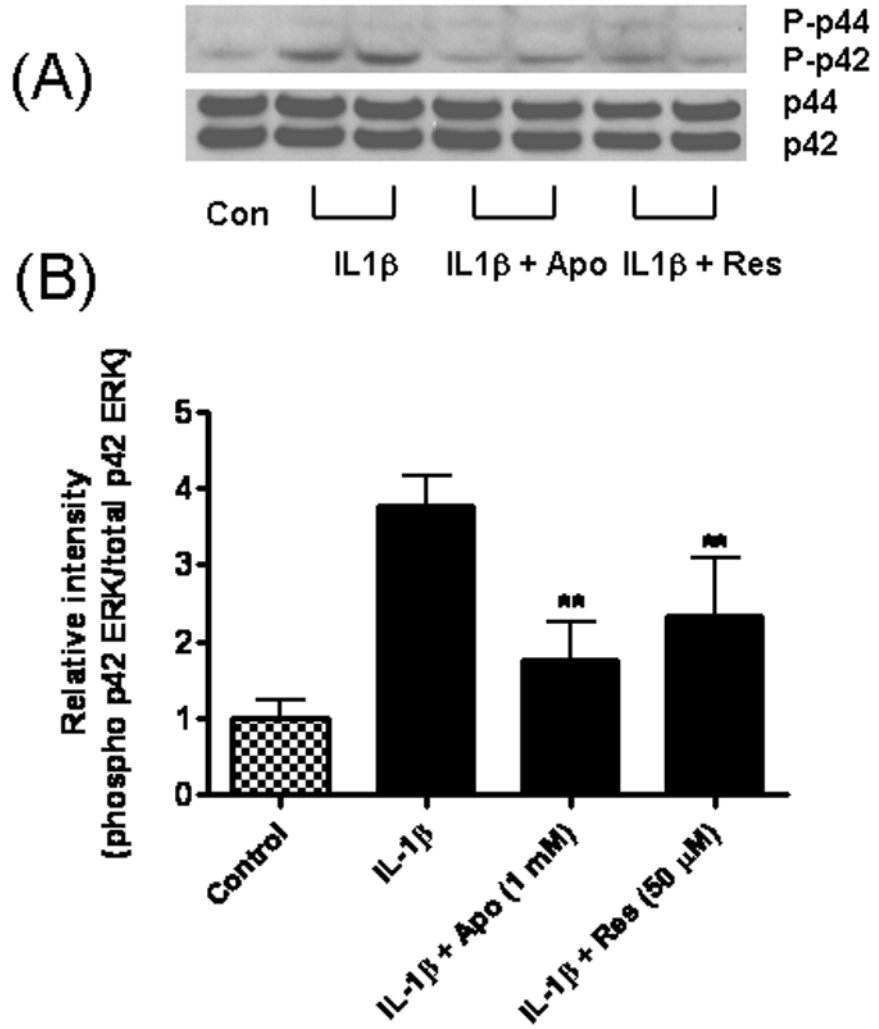

Fig 8.

Apocynin and resveratrol inhibited IL-1β-induced ERK1/2 phosphorylation in astrocytes. Astrocytes were treated with IL-1β (4 ng/ml), or IL-1β plus apocynin (1 mM) and Res (50 μM) for 10 min. (A) Cell lysate was used for Western blot analysis for phospho-ERK1/2 and total ERK1/2. (B) Relative intensities of phospho-p42 ERK against total p42 ERK. Results are mean ± SD from three independent experiments. **p<0.01 vs. IL-1β.