Down-regulation by prostaglandins of type-II phospholipase A_2 expression in guinea-pig alveolar macrophages: a possible involvement of cAMP

Daniel VIAL[1], Laurence ARBIBE, Nathalie HAVET, Claude DUMAREY, B. Boris VARGAFTIG and Lhousseine TOUQUI

Unité de Pharmacologie Cellulaire, Unité Associée Pasteur/INSERM U285, Institut Pasteur, 25 rue Dr. Roux 75015 Paris, France

We have demonstrated previously that isolated guinea-pig alveolar macrophages (AM) synthesize type-II phospholipase A_2 (PLA_2-II) through a tumour necrosis factor-α (TNF-α)-dependent process. This synthesis is enhanced by lipopolysaccharide (LPS) and accompanied by a release of prostaglandin E_2 (PGE_2) into the medium. Because agents elevating intracellular cAMP, such as PGE_2, have been shown to stimulate PLA_2-II expression in various cell types, we investigated the modulation of PLA_2-II synthesis by cAMP in AM. Surprisingly, incubation of AM with PGE_2, dibutyryl-cAMP, cholera toxin or rolipram (an inhibitor of specific cAMP-phosphodiesterase) inhibited both basal and LPS-stimulated PLA_2-II expression. The inhibitory effect of PGE_2 was observed at concentrations similar to those released by AM. Moreover, treatment of AM with either aspirin or neutralizing PGE_2 monoclonal antibody stimulated PLA_2-II synthesis. These effects were closely correlated with the ability of these agents to modulate TNF-α release, which was decreased by dibutyryl-cAMP and exogenous PGE_2, whereas neutralizing PGE_2 antibody markedly increased this release. Hence, in contrast to other cell systems, we report that: (i) agents elevating intracellular cAMP levels down-regulate both basal and LPS-induced PLA_2-II synthesis, (ii) prostaglandins exert a negative feedback effect on this synthesis, probably through an elevation of intracellular cAMP levels, and (iii) inhibition of TNF-α release may account, at least in part, for the down-regulation of PLA_2-II expression by endogenously produced prostaglandins and cAMP-elevating agents.

INTRODUCTION

Phospholipases A_2 (PLA_2, phosphatide 2-acylhydrolase, EC 3.1.1.4) catalyse the hydrolysis of ester bonds at the *sn*-2 position of membrane phospholipids and play a key role in the production of proinflammatory lipid-derived mediators, including lysophospholipids and arachidonic acid and its metabolites [1,2]. PLA_2s are divided into two major classes, the secretory or low-molecular mass (14–18 kDa) forms and the intracellular or high-molecular mass (40–85 kDa) forms [3–5], and were recently classified into nine types [5]. Among the secretory forms of PLA_2 ($sPLA_2$), the mammalian 'non-pancreatic' type-II PLA_2 (PLA_2-II) releases arachidonic acid and its proinflammatory metabolites from membranes of certain cell types, suggesting its possible involvement in the pathogenesis of inflammation [6–9].

In patients with adult respiratory distress syndrome, a positive correlation has been demonstrated between PLA_2-II levels measured in the bronchoalveolar lavage fluid and the severity of the disease [10,11]. Intratracheal injection of lipopolysaccharide (LPS) into blood-free isolated rat lungs stimulated PLA_2-II mRNA expression, suggesting that resident pulmonary cells such as alveolar macrophages (AM) may account for PLA_2-II production [12]. Indeed, we have recently reported that guinea-pig AM express PLA_2-II during adherence *in vitro* [13,14] and that this expression is increased several fold by 16 h exposure of AM to LPS [15]. Moreover, we showed that these cells are the major pulmonary source of PLA_2-II in LPS-induced acute lung injury in guinea-pigs and that the synthesis of this enzyme is mediated by tumour necrosis factor-α (TNF-α) through an autocrine process [15]. However, the intracellular events modulating PLA_2-II expression in AM have not been identified yet.

Agents elevating intracellular cAMP levels, such as dibutyryl-cAMP or forskolin, have been shown to stimulate PLA_2-II expression [16–24]. Hence, in various cell types, cAMP can act synergistically with interleukin-1 [17–19], TNF-α [18–20,24] or LPS [21–23] to increase PLA_2-II mRNA expression and protein synthesis.

The aim of the present study was to investigate the modulation of PLA_2-II expression by prostaglandins and cAMP-elevating agents in AM. Hence, in contrast to other cell systems, we report that: (i) agents elevating intracellular cAMP levels down-regulate both basal and LPS-induced PLA_2-II synthesis, (ii) prostaglandins exert a negative feedback action on this synthesis, probably through an elevation of intracellular cAMP levels, and (iii) inhibition of TNF-α release may account, at least in part, for the down-regulation of PLA_2-II expression by endogenously produced prostaglandins and cAMP-elevating agents.

MATERIALS AND METHODS

Materials

Male Hartley guinea-pigs were obtained from Elevages Saint-Antoine (Pleudaniel, France). RPMI 1640 culture medium, Dulbecco's medium, antibiotics and PBS without Ca^{2+} and Mg^{2+} were from Gibco (Bethesda Research Laboratories, Gaithersburg, MD, U.S.A.). Hepes, aspirin, actinomycin D, fatty acid-free BSA, leupeptin, aprotinin, L-glutamine, 2-mercaptoethanol, PMSF, cholera toxin and prostaglandin E_2 (PGE_2), were from Sigma (St. Louis, MO, U.S.A.). Dibutyryl-cAMP, rolipram and sodium pentobarbital were from Sanofi Laboratories (Montpellier, France), *Escherichia coli* 055:B5 LPS was from DIFCO Laboratories (Detroit, MI, U.S.A.) and fluorescent phospholipid [1-palmitoyl-2-(10-pyrenedecanoyl)-*sn*-glycero-monomethylphosphoglycerol] was from Interchim (Montluçon, France). Nylon membranes were purchased from Amersham (Les Ulis, France). Products for staining cytocentrifuge smears (modified May-Grünwald-Giemsa) were from Diff-Quik (Duedingen,

Abbreviations used: AM, alveolar macrophages; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; PGE_2, prostaglandin E_2; PLA_2, phospholipases A_2, PLA_2-II, type-II phospholipase A_2; $sPLA_2$, secretory phospholipase A_2; TNF-α, tumour necrosis factor-α.

[1] To whom correspondence should be addressed.

Switzerland) and [^{32}P]dCTP was from ICN Biochemicals France (Orsay, France).

Cell isolation and incubation procedures

Male Hartley guinea-pigs weighting 600–1000 g were anaesthetized by the intravenous injection of sodium pentobarbital (20 mg/kg). Twenty successive bronchoalveolar lavages were performed under sterile conditions with 5 ml aliquots of saline, containing 25 μg/ml of streptomycin and 25 units/ml of penicillin, which were injected with a plastic syringe through a polyethylene cannula inserted into the trachea. The cell suspensions were centrifuged at 475 ***g*** for 10 min at 25 °C and the pellets were washed twice with saline containing 25 μg/ml of streptomycin at 25 units/ml of penicillin. The washed cell pellets were resuspended in RPMI 1640 culture medium containing 50 μg/ml of streptomycin, 50 units/ml of penicillin, 2 mM L-glutamine, 10 mM Hepes (pH 7.2) and adjusted to 3×10^6 cells/ml. Differential counts were made on modified May-Grünwald-Giemsa-stained cytocentrifuge smears. The composition of the major cell types in the bronchoalveolar lavage fluids was macrophages 85.7 ± 6.3 %, eosinophils 8.6 ± 2.3 % and lymphocytes 5.7 ± 3.4 % (mean ± S.E.M., $n = 23$).

The cells, adjusted to 3×10^6 cells/ml, were allowed to adhere in 12-well plates for 1 h at 37 °C in 5 % CO_2/95 % air. At this stage, the cell population of adherent cells consisted of 95–99 % macrophages after the first hour of adhesion. The plates were then washed three times with medium. AM were incubated in serum-free RPMI for 16 h without (saline) or with LPS (25 μg/ml). In certain experiments, AM were preincubated with drugs or PGE_2 monoclonal antibody 1 h before the addition of LPS. Rolipram was dissolved in DMSO, PGE_2 in ethanol and aspirin, dibutyryl-cGMP and dibutyryl-cAMP in water. LPS and cholera toxin were dissolved in saline. In control experiments, AM were incubated with the solvents (DMSO or ethanol) alone at the same concentration (0.2 % final). All drugs were used at concentrations similar to those reported in the literature and had no toxic effect on AM. Neutralizing monoclonal PGE_2 antibody is a mouse immunoglubulin G 2B isotype previously shown to interact specifically with PGE_2 [25]. Another mouse monoclonal antibody (immunoglobulin G 2B isotype) directed against a protein of *Escherichia coli* was used as control.

Analysis of PLA_2-II mRNA levels

Cells were isolated and cultured as described above. Total RNA (10 μg/lane) was extracted as described by Chomczynski and Sacchi [26], electrophoresed on a 1 % agarose gel by the formaldehyde method [27] and then transferred on to nylon membranes. The blots were hybridized at 68 °C overnight as described by Church and Gilbert [28] using a ^{32}P-labelled (random priming) full-length guinea-pig PLA_2-II cDNA as a probe, and washed in $3\times$SSC ($1\times$SSC = 0.15 M NaCl/0.015 M sodium citrate) and 5 % SDS, followed by $1\times$SSC and 1 % SDS washes. Finally, blots were washed off and rehybridized with murine β-actin cDNA at 65 °C, as internal control.

It should be noted that the time exposure of the membrane to the film was longer in Figure 1(A) (2–3 days) than in Figures 1(B) and 1(C) (several hours) to better demonstrate the inhibitory effect of cholera toxin and db cAMP on basal $sPLA_2$ mRNA expression. If the time exposure of Figures 1(B) and 1(C) was similar to that of Figure 1(A), this would lead to the saturation of the autoradiogram by the signal of the LPS-induced $sPLA_2$ mRNA expression.

The same procedure was adopted for Figure 2. The time exposure was shorter in Figures 2(B) and 2(C) (right-hand side, by several hours) than that in Figure 2(A) (left-hand side, 2–3 days) to better demonstrate the stimulatory effect of aspirin and PGE_2 antibody on $sPLA_2$ mRNA expression in LPS-treated cells.

Preparation of cell lysates

At the end of the incubations, culture supernatants were harvested, centrifuged at 1500 ***g*** for 5 min at 4 °C to remove detached cells, and aliquots of 200 μl were stored at -20 °C until use. The culture dishes were kept in an ice-bath and adherent cells were washed and scraped with a rubber policeman in 0.5 ml of cold PBS containing 0.5 mM PMSF, 2 μg/ml leupeptin, 2 μg/ml aprotinin and 2 mM EDTA. Cells were then lysed by ultrasonication and kept at -20 °C until use.

Measurement of PLA_2-II activity

The measurement of $sPLA_2$ activity was carried out using the fluorimetric assay described by Radvanyi et al. [29] and shown to be selective for the $sPLA_2$ type. Furthermore, $sPLA_2$ activity measured in the pellet and supernatant of unstimulated and LPS-stimulated AM was totally blocked by the specific PLA_2-II inhibitor LY727311 [30] at 10 μM, indicating that $sPLA_2$ activity measured in AM corresponds to a PLA_2-II activity (data not shown).

Briefly, the fluorescent phospholipid was dried under nitrogen and suspended in ethanol at a concentration of 0.2 mM. Vesicles were prepared by mixing the ethanol solution of the fluorescent phospholipid with a buffer solution containing 50 mM Tris/HCl, 500 mM NaCl and 1 mM EGTA (pH 7.5). After 2 min of vigorous agitation, 960 μl of substrate solution were mixed in the cuvette with 10 μl of 10 % fatty acid-free BSA. Macrophage lysates and supernatants were maintained in an ice-cold bath throughout the experiment and aliquots (10–50 μg of proteins from cell lysates and 10–50 μl from supernatants) were introduced into the cuvettes and allowed to equilibrate at 37 °C for 1 min. The reactions were then initiated with 10 μl of $CaCl_2$ at a 10 mM final concentration in 4 mm × 10 mm disposable plastic cuvettes. The fluorescence measurements were performed with a Jobin et Yvon JY3D spectrofluorimeter equipped with a Xenon lamp and monitored using excitation and emission wavelengths of 345 and 398 nm, respectively, with a slitwidth of 4 nm.

Determination of TNF-α release

TNF-α bioactivity was measured by cytotoxicity on fibrosarcoma cells (WEHI 164 clone 13 line, kindly provided by Dr. F. J. Zijlstra, Erasmus University, Rotterdam, The Netherlands). Cells were grown in Dulbecco's medium supplemented with 10 % fetal calf serum (Boehringer, Mannheim, Germany) and antibiotics (1 % w/v gentamycin and 1 % w/v amphotericin B, Boehringer Mannheim) in an humidified atmosphere of 5 % CO_2. Cells (10^6/ml) were incubated for 3 h in the presence of 1 μg/ml actinomycin D. Aliquots of this cell suspension (50 μl/well containing 5×10^4 cells) were plated in 96-well flat-bottom microtitre plates (Nunclon Delta, Roskilde, Denmark) and incubated for 24 h with 50 μl samples or TNF-α stranded dilutions (10 to 10^4 pg/ml, recombinant human TNF-α; Bender-Wien, Vienna, Austria) in triplicate. The plates were further incubated for 24 h with 50 μl/well XTT {sodium 3′-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis(4-methoxy-6-nitro)-benzenesulphonic acid hydrate} labelling mixture prepared as recommended by the manufacturer (Cell Proliferation Kit II XTT, Boehringer Mannheim). Absorbance was measured in an automatic reader with a test wavelength of 490 nm and a reference

wavelength of 630 nm (Dynatech MR 5000, Marnes-La Coquette, France).

Determination of protein concentration and PGE_2 release

Protein concentration was measured as described by Smith et al. [31] using BSA as the standard. PGE_2 levels were measured by radioimmunoassay as previously described [32].

Control of cell viability

The cell viability was checked by the Trypan Blue dye exclusion test. Cell lysis was controlled by measuring the release of lactate dehydrogenase (LDH) activity in the medium using a commercial kit from Boehringer (Mannhein, Germany). No increase in LDH activity was observed in all experiments performed.

Calculations and statistical analysis

Data are expressed as means ± S.E.M. of separate experiments, and statistical analyses were performed using the unpaired Student's t test.

RESULTS

Effect of cAMP-elevating agents on PLA_2-II expression

We examined the effect of agents that increase intracellular cAMP levels on PLA_2-II expression in both unstimulated (basal expression) and LPS-stimulated AM. The addition of the permeable cAMP analogue dibutyryl-cAMP (100 μM) to the incubation medium markedly reduced PLA_2-II activity in both unstimulated and LPS-stimulated cells (Table 1). The structural analogue dibutyryl-cGMP (1.5 mM) had no effect (data not shown), suggesting that the inhibitory effect of dibutyryl-cAMP is not due to a non-specific action on cGMP-sensitive pathways. Similar results were obtained in AM treated with other agents that elevate intracellular cAMP concentrations, such as rolipram (1 μM), an inhibitor of cAMP-specific phosphodiesterase (Table 1), cholera toxin (50 ng/ml) (Table 1) and PGE_2 (100 ng/ml) (Table 1), known to stimulate adenylate cyclase activity in these cells [33,34].

Northern blot analysis indicated that the incubation of LPS-treated AM for 16 h with dibutyryl-cAMP, cholera toxin or PGE_2 prevented LPS-induced PLA_2-II mRNA expression (Figures 1B, 1C and 2A). Similar results were obtained when

Table 1 Effect of cAMP-elevating agents on PLA_2-II activity in AM

AM were treated with the drugs indicated or their solvents for 1 h and then incubated with LPS (25 μg/ml) or saline for 16 h. At the end of incubation, culture supernatants were harvested and centrifuged to remove detached cells. The measurement of PLA_2-II activity was performed in cell lysates and supernatants as indicated in the Materials and methods section. The results show the total (intra- plus extra-cellular) PLA_2-II activity, expressed as a percentage of control, and are the means ± S.E.M. of four to seven separate experiments. (a) db cAMP (dibutyryl-cAMP, 100 μM, $n = 5$), (b) rolipram (ROL, 1 μM, $n = 4$), (c) cholera toxin (TOX, 50 ng/ml, $n = 7$), (d) PGE_2 (100 ng/ml, $n = 4$). Statistical significance: $^{*}P < 0.05$, $^{**}P < 0.001$ compared with LPS-treated cells.

Addition	$sPLA_2$ activity (%)
(a)	
Control	100
db cAMP	15 ± 5
LPS	459 ± 116
LPS + db cAMP	97 ± 51*
(b)	
Control	100
ROL	57 ± 6
LPS	525 ± 71
LPS + ROL	260 ± 53*
(c)	
Control	100
TOX	20 ± 4
LPS	562 ± 165
LPS + TOX	76 ± 17*
(d)	
Control	100
PGE_2	33 ± 6
LPS	821 ± 30
LPS + PGE_2	179 ± 13**

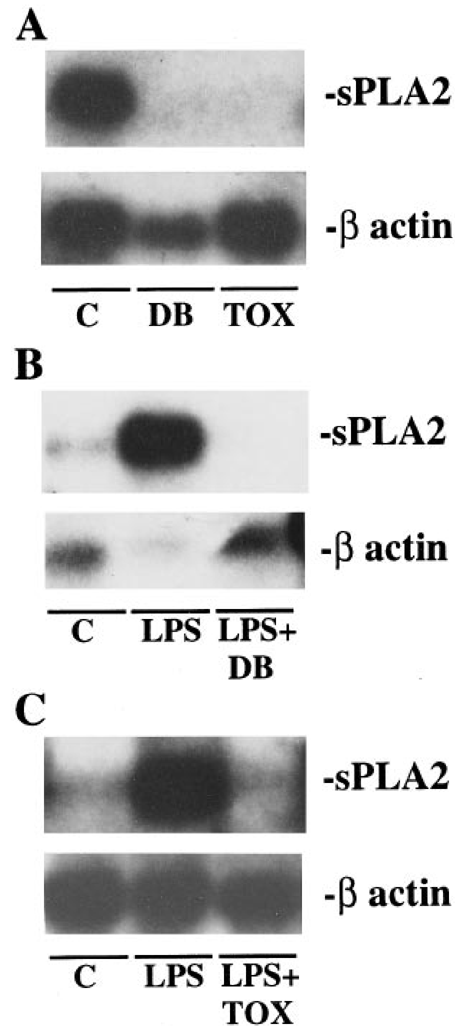

Figure 1 Effect of dibutyryl-cAMP and cholera toxin on PLA_2-II mRNA expression

Control and LPS-treated AM were incubated for 16 h with dibutyryl-cAMP (DB) (100 μM) or cholera toxin (TOX) (50 ng/ml) as indicated in Table 1. Total cellular RNA was then extracted from AM and Northern blotting (10 μg RNA/lane) was carried out as described in the Materials and methods section. Mouse β-actin was used as an internal control. 'C' corresponds to control cells. (**A**) Effect of cholera toxin or dibutyryl-cAMP on PLA_2-II mRNA expression in control cells. (**B**) and (**C**) inhibition of LPS-induced PLA_2-II mRNA expression by dibutyryl-cAMP and cholera toxin respectively. The Northern blots are representative of two to three independent experiments.

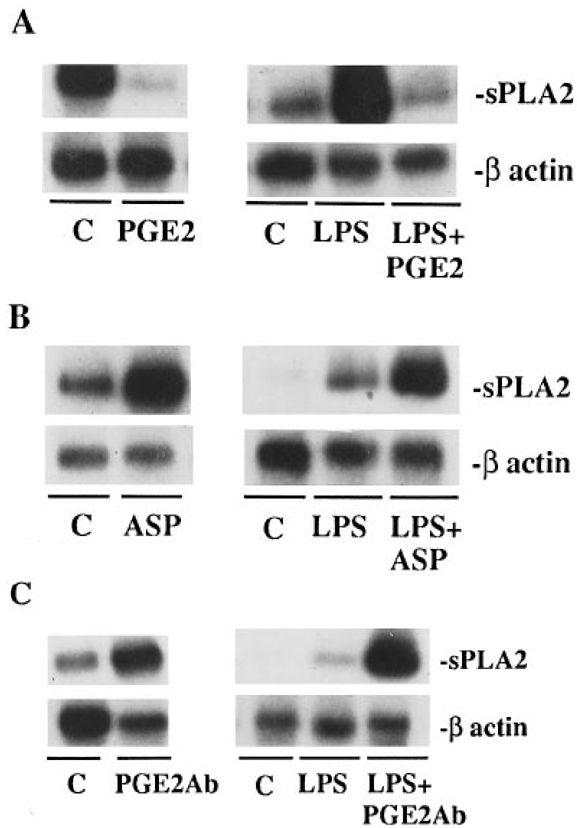

Figure 2 Effect of PGE_2, aspirin and neutralizing PGE_2 antibody on PLA_2-II mRNA expression

Control and LPS-treated AM were incubated for 16 h with PGE_2 (100 ng/ml), aspirin (ASP) (200 μM) or neutralizing PGE_2 antibody (PGE2Ab) (1 μg/ml) as indicated in Table 2. Total cellular RNA was then extracted from AM and Northern blotting (10 μg RNA/lane) was carried out as described in the Materials and methods section. Mouse β-actin was used as an internal control. 'C' corresponds to control cells. (**A**) Effect of PGE_2 on PLA_2-II mRNA expression in control and LPS-treated cells. (**B**) and (**C**) effect of aspirin and neutralizing PGE_2 antibody respectively. The Northern blots are representative of two to three independent experiments.

unstimulated AM were incubated with these agents (Figures 1A and 2A).

Effect of endogenously produced prostaglandins on PLA_2-II expression

Because PGE_2 down-regulated PLA_2-II expression in AM, we investigated the role of endogenous prostaglandins. As shown in Figure 2(B) and Table 2, aspirin (200 μM) increased PLA_2-II expression in both unstimulated and LPS-stimulated AM, indicating that cyclo-oxygenase products may exert a negative feedback on PLA_2-II synthesis. In agreement, a murine monoclonal antibody against PGE_2 increased PLA_2-II expression both in unstimulated and LPS-stimulated AM (Figure 2C and Table 2). We next examined whether the levels of PGE_2 released by AM were sufficient to inhibit PLA_2-II synthesis. Unstimulated AM released low levels of PGE_2 (0.5 ± 0.2 ng/ml, $n = 4$) which remained constant during the 16 h interval of incubation. After LPS addition, the levels of PGE_2 remained constant and similar to control values up to 6 h after the challenge. Thereafter, the release of PGE_2 increased to reach 5 ± 3 ng/ml ($n = 4$) after 16 h of stimulation. These concentrations are compatible with those of exogenous PGE_2 required for inhibiting PLA_2-II synthesis (Table 3). In another set of experiment, endogenous prostaglandins were eliminated by exposing the cells to aspirin before adding PGE_2. Under these conditions, the stimulatory effect of aspirin on PLA_2-II synthesis was completely reversed by concentrations of exogenous PGE_2 as low as 0.5 ng/ml (Table 3).

Taken together, these results show that endogenously produced prostaglandins, and particularly PGE_2, exert a negative feedback on PLA_2-II synthesis.

Table 2 Effect of aspirin and neutralizing PGE_2 antibody on PLA_2-II activity in AM

AM were incubated for 16 h with aspirin or mouse PGE_2 monoclonal antibody before measurement of PLA_2-II activity. For LPS-treated cells, AM were pretreated with aspirin and neutralizing PGE_2 antibody 1 h before the addition of LPS (25 μg/ml). The results show the total (intra- plus extra-cellular) PLA_2-II activity, expressed as a percentage of control, and are the means $\pm$ S.E.M. of four separate experiments. (a) Aspirin (ASP, 200 μM, $n = 4$), (b) mouse PGE_2 monoclonal antibody (PGE_2 Ab, 1 μg/ml, $n = 4$), control mouse monoclonal antibody (IgG_2B, 1 μg/ml). Statistical significance: $^*P < 0.01$ compared with LPS-treated cells.

Addition	$sPLA_2$ activity (%)
(a)	
Control	100
ASP	319 ± 93
LPS	456 ± 58
LPS + ASP	$1085 \pm 175^*$
(b)	
Control	100
PGE_2 Ab	380 ± 43
IgG_2B	95 ± 11
LPS	450 ± 76
LPS + PGE_2 Ab	$995 \pm 103^*$
LPS + IgG_2B	364 ± 31

Table 3 Concentration-dependent effect of exogenous PGE_2 on PLA_2-II activity in AM

(a) AM were preincubated for 1 h with different concentrations of PGE_2 (0.5, 1 or 10 ng/ml) and then incubated with LPS for 16 h before the measurement of PLA_2-II activity. Unpaired Student's *t* test was for comparison with LPS-treated cells. (b) Combined effect of aspirin and PGE_2 on PLA_2-II activity. AM were pretreated with aspirin (ASP, 200 μM) for 1 h and then incubated with various concentrations of PGE_2 (0.5, 1 or 10 ng/ml) for 16 h. Unpaired Student's *t* test was used for comparison with aspirin-treated cells. The results show the PLA_2-II activity expressed as a percentage of control, and are the means $\pm$ S.E.M. of three experiments. Statistical significance: $^*P < 0.01$.

Addition	$sPLA_2$ activity (%)
(a)	
Control	100
PGE_2 (0.5 ng/ml)	60 ± 9
PGE_2 (1 ng/ml)	43 ± 9
PGE_2 (10 ng/ml)	25 ± 6
LPS	397 ± 8
LPS + PGE_2 (0.5 ng/ml)	282 ± 52
LPS + PGE_2 (1 ng/ml)	$184 \pm 32^*$
LPS + PGE_2 (10 ng/ml)	$120 \pm 35^*$
(b)	
Control	100
ASP	340 ± 34
ASP + PGE_2 (0.5 ng/ml)	$110 \pm 5^*$
ASP + PGE_2 (1 ng/ml)	$75 \pm 7^*$
ASP + PGE_2 (10 ng/ml)	$48 \pm 9^*$

Table 4 Modulation of TNF-α release by dibutyryl-cAMP, PGE_2 and neutralizing PGE_2 antibody

Control and LPS-treated AM were incubated with the compounds indicated for 16 h. Culture supernatants were harvested, centrifuged to remove detached cells, and samples of 200 μl were used to measure TNF-α release as described in the Materials and methods section. (a) Dibutyryl-cAMP (db cAMP, 100 μM, $n = 5$), (b) PGE_2 (100 ng/ml, $n = 4$), (c) PGE_2 antibody (PGE_2 Ab, 1 μg/ml, $n = 4$). The results are the means $\pm$ S.E.M. of four to five separate experiments. Statistical analysis was performed using the unpaired Student's t test in comparison with corresponding controls. Statistical significance: $^{*}P < 0.05$.

Addition	TNF-α release (ng/ml)
(a)	
Control	27 ± 8
db cAMP	$5 \pm 2^{*}$
LPS	310 ± 60
LPS + db cAMP	$91 \pm 27^{*}$
(b)	
Control	33 ± 12
PGE_2	$1.6 \pm 0.2^{*}$
LPS	191 ± 26
LPS + PGE_2	$75 \pm 18^{*}$
(c)	
Control	16 ± 4
PGE_2 Ab	$55 \pm 10^{*}$
LPS	168 ± 33
LPS + PGE_2 Ab	$337 \pm 48^{*}$

Modulation of TNF-α release by PGE_2, dibutyryl-cAMP and monoclonal PGE_2 antibody

As we have previously demonstrated that the synthesis of PLA_2-II in AM is mediated by TNF-α [15], we investigated whether the effect of PGE_2 and cAMP-elevating agents on PLA_2-II expression is due to the inhibition of TNF-α release. Table 4 shows that dibutyryl-cAMP and PGE_2 inhibited TNF-α release in unstimulated (about 80 % and 90 % respectively) and LPS-stimulated (70 % and 60 % respectively) AM. In addition, when monoclonal PGE_2 antibody was added to the medium, a marked increase in TNF-α release was observed in both unstimulated (3.5-fold) and LPS-treated (2-fold) cells (Table 4), indicating that endogenously produced PGE_2 exerts a negative feedback on TNF-α release.

DISCUSSION

We report here that agents elevating intracellular cAMP concentrations suppress PLA_2-II synthesis in unstimulated and LPS-stimulated AM. This inhibitory effect contrasts with that observed in other cell types, in which raising cytosolic cAMP levels stimulated PLA_2-II expression and potentiated that induced by proinflammatory stimuli [16–24]. Hence, in various cell types, cAMP can act synergistically with interleukin-1 [17–19], TNF-α [18–20,24] or LPS [21–23] to increase PLA_2-II mRNA expression and protein synthesis. Here, we show that agents that directly increase cAMP levels, such as the structural cAMP analogue dibutyryl-cAMP or the phosphodiesterase inhibitor rolipram, inhibit PLA_2-II expression in guinea-pig AM. Similar results are observed with cholera toxin, which stimulates adenylate cyclase activity through activation of the G-protein α_s subunit, and PGE_2, previously shown to raise the intracellular cAMP level in these cells [33,34].

Moreover, aspirin increased PLA_2-II synthesis in both unstimulated and LPS-stimulated AM, indicating that cyclo-oxygenase products can exert a negative feedback on PLA_2-II production. The use of neutralizing monoclonal PGE_2 antibody allowed us to determine that PGE_2 is largely responsible for this inhibitory effect. In agreement, when exogenous PGE_2 was added at concentrations similar to those produced by AM, a decrease in PLA_2-II expression was observed, overcoming the stimulating effect of aspirin. This suggests that the amounts of PGE_2 produced by AM are sufficient to regulate negatively PLA_2-II expression, although the participation of other cyclo-oxygenase products in this process cannot be excluded.

The involvement of PGE_2 in the inhibition of PLA_2-II expression has been previously reported in guinea-pig peritoneal macrophages [35]. However, the role of cAMP in this process remained unclear, as cAMP-elevating agents failed to inhibit PLA_2-II expression in this cell type.

This regulation of PLA_2-II expression by endogenous PGE_2 in AM differs from that reported in rat mesangial cells [19]. Indeed, in this case, exogenously added PGE_2 stimulated PLA_2-II expression and the cyclo-oxygenase inhibitor indomethacin suppressed interleukin-1- and TNF-α-stimulated PGE_2 synthesis without affecting PLA_2-II expression, thus excluding endogenous prostaglandins as mediators of interleukin-1- and TNF-α-induced PLA_2-II synthesis in mesangial cells [19].

We have recently shown [15] that the induction of PLA_2-II expression in AM is dependent on TNF-α in an autocrine fashion. Previous studies indicate that PGE_2 and agents elevating cAMP levels inhibited both *in vitro* [36–39] and *in vivo* [40] the release of TNF-α, probably by inhibiting TNF-α mRNA expression, and that cyclo-oxygenase inhibitors enhanced this expression [36]. Our experiments show that dibutyryl-cAMP and PGE_2 inhibit the release of TNF-α and that neutralizing monoclonal PGE_2 antibody increases this release. These results suggest that the decrease in PLA_2-II production produced by PGE_2 and cAMP-elevating agents may be due, at least in part, to the reduction in TNF-α release. However, other mechanisms can explain the inhibitory effect of cAMP-elevating agents on PLA_2-II expression in AM. For example, cAMP may act by modulating the activation of transcription factors involved in PLA_2-II gene expression. Moreover, in the monoblastic THP-1 cell line, it has been reported that cAMP activates the proteolytic cleavage of membrane TNF-α receptors [41], diminishing the number of TNF-α receptors on the membrane. Hence, cAMP can also regulate PLA_2-II expression in AM through a modulation of TNF-α receptors. Binding studies with TNF-α are necessary to investigate this hypothesis, but unfortunately guinea-pig TNF-α is not available and TNF-α from other species does not cross-react with guinea-pig TNF-α [15].

The physiopathological relevance of this study is linked to the fact that AM are *in vivo* the major pulmonary source of PLA_2-II in LPS-induced acute lung injury [15]. The increase in prostaglandin level in the alveolar space during the inflammatory reaction [42–44] may play an important role in the down-regulation of PLA_2-II synthesis, thus avoiding its overexpression by inflamed lungs.

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