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Selective phosphodiesterase inhibitors modulate the activity of alveolar macrophages from sensitized guinea-pigs

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Selective phosphodiesterase inhibitors modulate the activity of alveolar macrophages from sensitized guinea-pigs. N. Germain, B. Bertin, A. Legendre, B. Martin, V. Lagente, A. Payne, E. Boichot. ©ERS Journals Ltd 1998.

ABSTRACT: The aim of this study was to investigate the effects of selective phosphodiesterase (PDE)3 and PDE4 inhibitors on arachidonate release by alveolar macrophages from sensitized and challenged guinea-pigs.

Guinea-pigs were sensitized and challenged with ovalbumin administered by aerosol. Bronchoalveolar lavage was performed 48 h later and the PDE and cyclic adenosine monophosphate (cAMP) contents of or the arachidonate release from alveolar macrophages, stimulated *in vitro* with *N*-formyl-Met-Leu-Phe (fMLP), were evaluated.

PDE3 and PDE4 activities were detected in preparations of macrophage lysate from sensitized challenged and sensitized control animals. Oral pretreatment, prior to antigen challenge in sensitized guinea-pigs, with rolipram or Ro 20-1724 (PDE4 inhibitors) but not milrinone (PDE3 inhibitor) significantly reduced the arachidonate release from alveolar macrophages. *In vitro* incubation of alveolar macrophages from challenged guinea-pigs with Ro 20-1724 or the cAMP analogue dibutyryl cAMP (db-cAMP) but not milrinone or the cyclic guanosine monophosphate (cGMP) analogue 8-bromo-cGMP (8-br-cGMP) significantly reduced arachidonate release. Incubation of the cells with a combination of milrinone plus rolipram or Ro 20-1724 elicited a marked and significant reduction in arachidonate release by alveolar macrophages stimulated with fMLP.

In conclusion, these data show that phosphodiesterase-4 isoenzyme may regulate the release of inflammatory mediators such as arachidonate from macrophages through an increase in intracellular cyclic adenosine monophosphate. This suggests that phosphodiesterase-4 inhibitors have potential in the treatment of inflammatory disorders of the lung.

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The cyclic nucleotides cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) are important second messengers in cell function. The concentration of intracellular cAMP increases either as a consequence of receptor-triggered adenylyl cyclase activation or by decreased activity of phosphodiesterase (PDE), which regulates the breakdown of cAMP and cGMP. PDE are a growing group of enzymes, classified into seven distinct families (PDE1–PDE7) with several subtypes and splice variants (for recent reviews, see [1 , 2]). Among them, PDE3, PDE4 and PDE7 appear to be the most important in the regulation of cAMP. In the majority of inflammatory cells, the low Michaelis constant (*k*M) cAMP-specific members of the PDE4 family are the most prominently expressed and, thus, have attracted attention as a pharmacological target in the field of inflammatory drug development [3–5]. Selective PDE4 inhibitors such as rolipram and Ro 20-1724 have been shown to inhibit several leukocyte functions, including inflammatory mediator-release [5].

In mononuclear cells, PDE3 is also involved in the regulation of cAMP levels. Indeed, the selective PDE3 inhibitor milrinone elicits a moderate inhibition of arachidonate release from these cells [6] and appears to synergize with inhibitors of PDE4 to reduce proliferation [7]. In macrophages, in the presence of the adenylyl cyclase activator, prostaglandin E2 (PGE2), PDE3 inhibitors are as effective as PDE4-selective drugs in the inhibition of tumour necrosis factor-α release [8]. Therefore, HATZELMANN *et al.* [9] proposed that PDE3–PDE4 synergism may reduce the risk of side-effects caused by each selective inhibitor alone, since it is plausible that the required doses of dual inhi-bitors of PDE3 and PDE4 isoenzymes will be markedly lower than those of selective inhibitors alone.

Alveolar macrophages play a major role in lung defence. In addition, they are the main cell type involved in the tissue injury associated with inflammatory disease in the lung, including asthma and acute respiratory distress syndrome (for review, see [10]). Indeed, alveolar macrophages are able to release several inflammatory mediators, including cytokines, growth factor and arachidonic acid metabolites, in various pathophysiological situations (for review, see [11]). Bronchial hyperresponsiveness is associated with enhanced activity of alveolar macrophages collected from sensitized and antigen-challenged guinea-pigs or naive guinea-pigs exposed to an aerosol of substance P [12, 13].

The present study was undertaken in order to investigate the effects of selective PDE3 and PDE4 inhibitors on arachidonate release by alveolar macrophages from sensitized and challenged guinea-pigs. Moreover, the involvement of intracellular cAMP was analysed and the cAMP–PDE isoenzyme activity profile in these cells identified.

Materials and methods

Materials

The following drugs were used: bovine serum albumin (BSA), *N*-formyl-Met-Leu-Phe (fMLP), 3-isobutyl-1-methylxanthine (IBMX), ethylenediaminetetra-acetic acid (EDTA), ovalbumin (OA; chicken egg, grade V), milrinone, N^6,2'-*O*-dibutyryladenosine 3',5'-cyclic monophosphate (db-cAMP), 8-bromoguanosine 3',5'-cyclic monophosphate (8-br-cGMP), tris-hydroxymethyl-amino methane (Tris), $MgCl_2$, dithiothreitol, aprotinin, benzamidine, soybean trypsin inhibitor and bacitracin were obtained from Sigma (St Louis, MO, USA); racemic rolipram was synthesized at the Institut de Recherche Jouveinal Fresnes, France; Ro 20-1724 was from RBI (Natick, MA, USA); phosphate-buffered saline (PBS) was from Gibco (Cergy-Pontoise, France); ^{3}H-arachidonic acid was from Amersham (Les Ulis, France); urethane (ethylcarbamate) was from Prolabo (Gradignan, France); ^{3}H-AMP and ^{3}H-cGMP were from Dupont de Nemours; and trichloroacetic acid (TCA) was obtained from Merck (Darmstadt, Germany).

Sensitization procedure and challenge

Pathogen-free male Hartley guinea-pigs (300–350 g; Charles River, St Aubin les Elboeuf, France) were used throughout the study. Guinea-pigs were sensitized and challenged as described previously [14]. In brief, they were placed in a Plexiglass chamber (30×50×30 cm) and exposed twice for 30 min to an aerosol of 2 mg·mL^{-1} OA in saline (NaCl, 0.9%), at an interval of 48 h. The aerosol was generated by a Devilbiss ultrasonic nebulizer (ULTRA-NEB 99; Somerset, PA, USA). At 15–20 days after the initial sensitization procedure, the guinea-pigs were challenged by exposure to five successive concentrations of OA: 10 µg·mL^{-1}, 100 µg·mL^{-1}, 1 mg·mL^{-1}, 5 mg·mL^{-1} and 10 mg·mL^{-1} for 15 min each. Control guinea-pigs were exposed to a saline solution for an equivalent period.

Bronchoalveolar lavage

Bronchoalveolar lavage (BAL) was performed 48 h after OA challenge. Guinea-pigs were anaesthetized with urethane (1.2 g·kg^{-1}, *i.p.*), the trachea was cannulated and 5 mL of a saline solution (37°C) containing 2.6 mM EDTA was instilled into the lungs with a 5-mL syringe. The lavage fluid was recovered by gentle aspiration. This procedure was repeated 10 times and the fluids were combined. Total fluid recovery was >85% of the injected volume. The lavage fluid was adjusted to 50 mL with PBS. After centrifugation at 350×*g* for 10 min, the cell pellet was resuspended in 10 mL PBS. Total cell counts were determined on Malassez chambers (Poly Labo, Strasbourg, France) by means of optical microscopy.

Isolation and identification of phosphodiesterase

Freshly prepared alveolar macrophages were isolated, after adhesion on plastic Petri dishes for 60 min at 37°C, resuspended in buffer containing 10 mM Tris, 1 mM $MgCl_2$, 1 mM dithiothreitol, 2 U·mL^{-1} aprotinin, pH 7.5, and sonicated using a Labsonic sonicator (Bomem/Hartmann et Braun, Quebec, QC, Canada). After centrifugation at 40,000 ×*g* for 30 min, the supernatant was frozen at -20°C for subsequent purification of isoenzymes. PDE isoenzyme separation was performed according to the method of LAVAN *et al.* [15] using anion-exchange (Mono-Q; Pharmacia Biotech, Saclay, France) column chromatography in association with fast protein liquid chromatography (FPLC; Biocad; Perspective Biosystem, Perkin Elmer, Norwalk, CT, USA). The column was equilibrated with a 60-mL bed volume of buffer containing 20 mM bis-Tris, pH 6.5, 10 mM EDTA, 2.5 mM dithiothreitol and a protease inhibitor mixture containing 2 mM benzamidine, 2 µg·mL^{-1} soybean trypsin inhibitor, 100 µg·mL^{-1} bacitracin and 50 µM phenylmethylsulphonyl fluoride (PMSF). Enzyme extracts were loaded on the column, eluted with 100 mL of a linear (0–1 M) NaCl gradient and 1.5-mL fractions collected over the entire gradient. To stabilize the enzyme, 0.1 mg BSA was added to each fraction. The activities of individual PDE isoenzymes were determined in 96-well microplates using 0.1 µM cAMP and 74 kBq·mL^{-1} (2 µCi·mL^{-1}) ^{3}H-cAMP in 40 mM Tris-HCl, 5 mM $MgCl_2$, 4 mM 2-mercaptoethanol in distilled water. All fractions were assayed in the absence or presence of 30 µM rolipram or SKF 94836. The reaction was stopped with 25 µ 0.4% TCA and the entire fraction was transferred into 96-well plates (Loprodyne; Pall Filtron, Saint Germain en Laye, France) containing 50 mg $Al(OH)_3$. ^{3}H-AMP was separated from ^{3}H-cAMP with $AL(OH)_3$ after elution with 1 M NaOH. The results are expressed as counts per minute (cpm) of PDE activity in each fraction.

Measurement of cyclic adenosine monophosphate levels

Alveolar macrophages were distributed in 12-well plates (Falcon, Le Pont de Claix, France) (at 1.5×10^6 cells·well^{-1}) and allowed a stabilization time of 60 min at 37°C in the presence of 5% foetal serum cell (FCS), followed by 60 min at 37°C without FCS. In some experiments, the cells were incubated at 37°C for 20 min with 200 µM IMBX. The reactions were stopped by the addition of 0.5 mL 0.1 N HCl at 4°C. The supernatant was immediately frozen at -80°C until cAMP determinations could be performed. The levels of cAMP were determined by radioimmunoassay using ^{125}I–cAMP (RIANEN; Dupont, Wilmington, USA).

Protocol

In the first set of experiments, guinea-pigs were treated orally with rolipram (3 mg·kg^{-1}), Ro 20-1724 (30 mg·kg^{-1}) or milrinone (30 mg·kg^{-1}), 24 h and 3 h before OA challenge. The protocol and doses of selective PDE inhibitors used have previously been shown to be effective in the reduction of recruitment of eosinophils in sensitized guinea-pigs [16].

In the second set of experiments, alveolar macrophages from sensitized challenged or sensitized control guinea-pigs were incubated *in vitro* for 30 min with rolipram (10 μM), Ro 20-1724 (10 μM), milrinone (10 μM), db-cAMP (1 mM) or 8-br-cGMP (1 mM). These concentrations of selective PDE inhibitors have previously been shown to be effective in the reduction of arachidonate release from human mononuclear cells [6].

Alveolar macrophage activation and arachidonate release

Alveolar macrophages were separated by adherence, for 1 h at 37°C, to plastic Petri dishes (2×10^6 cells·2 mL^{-1}·35 mm·$dish^{-1}$ (Falcon)) in an atmosphere of 5% CO_2 and 100% humidity. For arachidonic acid incorporation, macrophages were labelled with ^{3}H-arachidonic acid (22 kBq (0.6 μCi)·2×10^6 $cells^{-1}$). One hour later, the supernatant was discarded and the cells were washed three times with PBS containing 0.2% BSA. After 30 min resting times, macrophages were stimulated with 1 μM fMLP for 10 min. For the *in vitro* protocol, macrophages were incubated with various compounds for 30 min and then stimulated with 1 μM fMLP for 10 min. Samples were then centrifuged for 3 min at 1,250×*g*, 0.4 mL of supernatant was added to 2 mL of scintillation cocktail (Packard Instrument, Meriden, CT, USA) in Pico vials (Packard Instrument) and samples were counted in a liquid scintillation analyser (Packard Instrument).

Data analysis

Release of ^{3}H-arachidonate was expressed as a percentage of the cpm recovered in the supernatant compared to the control values (cells without stimulation by fMLP). Results are expressed as means±SEM. For the cAMP levels, the results are expressed as means (fM·10^6 $cells^{-1}$)± SEM. Analyses for statistical significance were performed using the nonparametric Mann–Whitney U-test.

Results

Characterization of cyclic adenosine monophosphate dependent phosphodiesterase activity in alveolar macrophages

To identify the cAMP-dependent PDE isoenzymes present in alveolar macrophages from sensitized guinea-pigs, supernatants of cell lysates were applied to an anion exchange column and the PDE activity eluted with a linear NaCl gradient. A broad peak of PDE activity eluting between 400 and 650 mM NaCl was noted in alveolar macrophages from both saline-exposed (fig. 1a) and OA-challenged (fig. 1b) sensitized guinea-pigs. Two peaks of PDE4 activity, at 450–500 mM and at 525–550 mM NaCl, were detected in both preparations, as shown by the marked inhibitory activity of 30 μM rolipram (fig. 1a and b). Similarly, a peak of PDE3 activity was noted at 625–650 mM NaCl, as demonstrated by the inhibitory activity of 30 μM SKF 94836. No marked difference in cAMP-hydrolysing PDE activity was found between saline-ex-posed and OA-challenged guinea-pigs (fig. 1a and b).

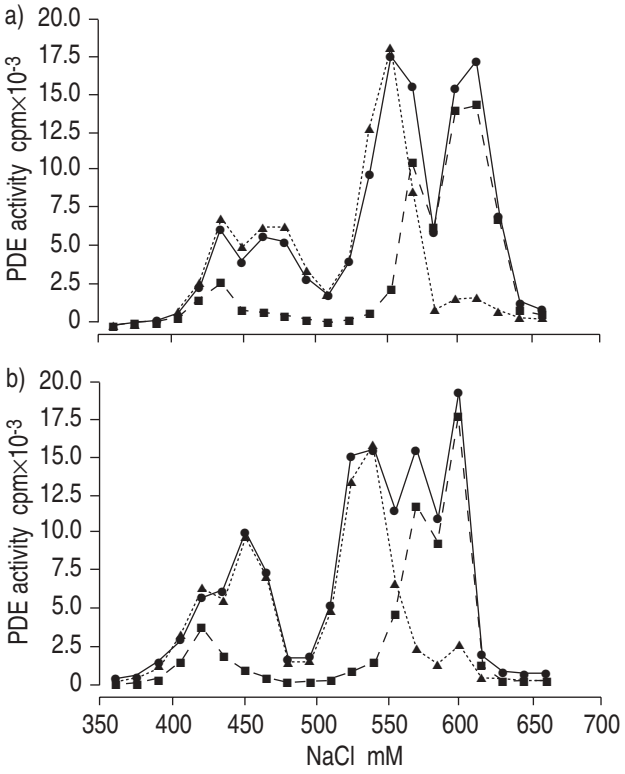

Fig. 1. – Elution profile of cyclic adenosine monophosphate (cAMP)-dependent phosphodiesterase (PDE) activity of guinea-pig alveolar macrophages. Alveolar macrophages were collected from a) saline-exposed or b) ovalbumin (OA)-challenged, sensitized guinea-pigs. The macrophages were lysed and the supernatant fraction was applied to an anion-exchange column. The protein was eluted with a linear NaCl gradient containing 0.1 mM cAMP (control; ●); 30 μM rolipram (■); or 30 μM SK&F 94836 (▲). cpm: counts per minute.

Effect of ovalbumin challenge on intracellular cyclic adenosine monophosphate levels in alveolar macrophages

The levels of cAMP were evaluated 1 h and 48 h following exposure of sensitized guinea-pigs to either saline or OA. No significant change in cAMP levels was noted in alveolar macrophages incubated with or without 200 μM IBMX, 1 h after challenge in both groups of guinea-pigs (fig. 2). In contrast, at 48 h, a marked and significant increase in the intracellular level of cAMP was observed in macrophages from OA-challenged guinea-pigs compared with the cells from control guinea-pigs (fig. 2). The changes in cAMP levels were also observed after incubation of alveolar macrophages with 200 μM IBMX. In these conditions the level of cAMP was considerably elevated at 48 h, particularly in alveolar macrophages from OA-challenged guinea-pigs (fig. 2).

Effect of oral treatment with phosphodiesterase inhibitors on arachidonate release from alveolar macrophages after ovalbumin challenge in aerosol-sensitized guinea-pigs

OA challenge in aerosol-sensitized guinea-pigs elicited a marked and significant increase in arachidonate release from alveolar macrophages stimulated *in vitro* with (1 μM) fMLP (figs. 3 and 4). Pretreatment at 24 h and 3 h

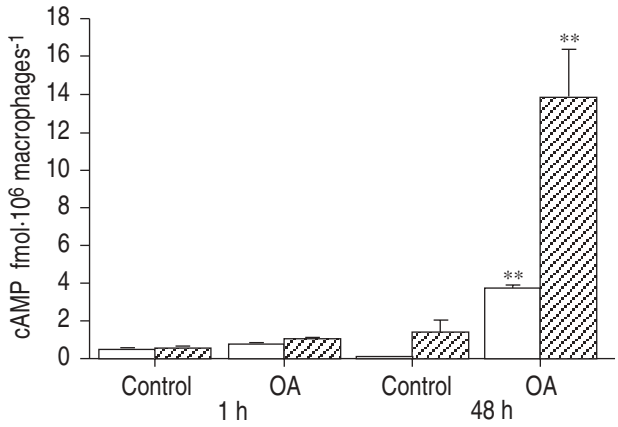

Fig. 2. – Intracellular cyclic adenosine monophosphate (cAMP) levels of alveolar macrophages from sensitized guinea-pigs, 1 h or 48 h following saline-exposure (control) or ovalbumin (OA) challenge. In some experiments, the cells were incubated with 200 μM 3-isobutyl-1-methylxanthine (IBMX; ▨). The results are expressed in fmol of cAMP in 10^6 macrophages±SEM. **: p<0.01 compared with control (n=6–7).

before OA challenge with rolipram (3 mg·kg^{-1}) or Ro 20-1724 (30 mg·kg^{-1}) significantly reduced the increase in arachidonate release after *in vitro* stimulation of the macrophages with fMLP. No significant changes in the arachidonate release were noted in saline-exposed guinea-pigs treated with either rolipram or Ro 20-1724. Pretreatment of OA-challenged guinea-pigs with milrinone (30 mg·kg^{-1}) moderately but nonsignificantly reduced the fMLP-induc-ed arachidonate release (fig. 3).

Effect of in vitro *incubation with phosphodiesterase inhibitors on arachidonate release from alveolar macrophages from sensitized guinea-pigs*

No significant inhibition of fMLP-induced arachidonate release from alveolar macrophages collected from saline-exposed guinea-pigs was noted upon *in vitro* incubation of

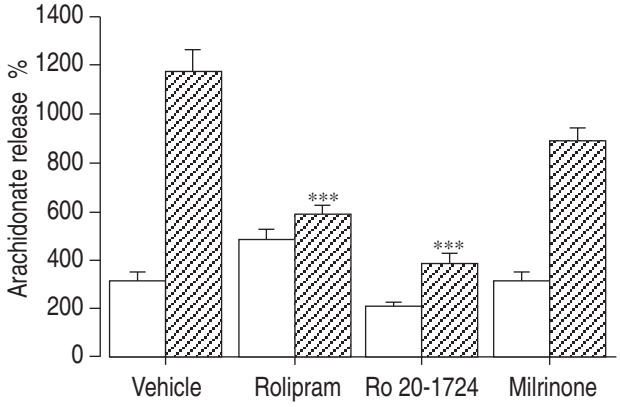

Fig. 3. – Effect of the selective phosphodiesterase-4 (PDE4) inhibitors rolipram (3 mg·kg^{-1}) and Ro 20-1724 (30 mg·kg^{-1}) and the selective PDE3 inhibitor milrinone (30 mg·kg^{-1}) on arachidonate release by *N*-formyl-Met-Leu-Phe (fMLP)-stimulated alveolar macrophages collected from saline-exposed (▭) or ovalbumin (OA)-challenged (▨) sensitized guinea-pigs. Guinea-pigs were treated orally with PDE inhibitors 24 h and 3 h before challenge and the alveolar macrophages were collected at 48 h. Results are expressed as means (percentage of release) of arachidonate±SEM. ***: p<0.001 compared with vehicle (n=6–7).

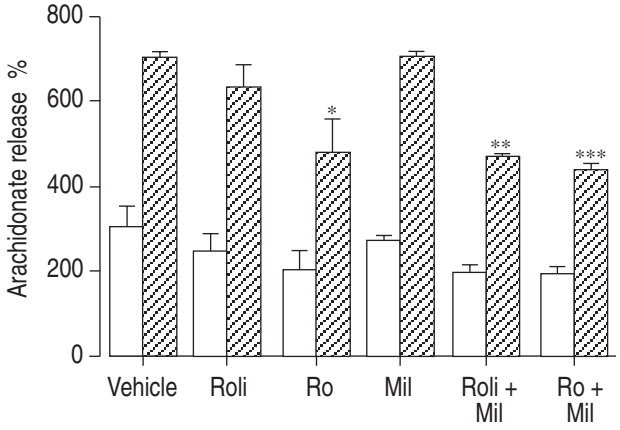

Fig. 4. – Effect of the selective phosphodiesterase-4 (PDE4) inhibitors rolipram (Roli; 10^{-5} M) and Ro 20-1724 (Ro; 10^{-5} M), and the selective PDE3 inhibitor milrinone (Mil 10^{-5} M) and their combinations on the arachidonate release by *N*-formyl-Met-Leu-Phe (fMLP)-stimulated alveolar macrophages collected from saline-exposed (▭) or ovalbumin (OA)-challenged (▨) sensitized guinea-pigs. Alveolar macrophages were collected at 48 h following challenge and incubated *in vitro* for 30 min with the compounds. Results are expressed as means (percentage of release) of arachidonate±SEM from four experiments performed in triplicate. *: p<0.05; **: p<0.01; ***: p<0.001 compared with vehicle.

the cells with the PDE inhibitors rolipram, Ro 20-1724 or milrinone, the cAMP analogue db-cAMP or the cGMP analogue 8-br-cGMP (fig. 4).

Incubation of alveolar macrophages from OA-challenged guinea-pigs with rolipram (10^{-5} M) or milrinone (10^{-5} M) did not significantly reduce the fMLP-induced increase in arachidonate release from these cells (fig. 4). Ro 20-1724 (10^{-5} M) slightly and significantly reduced arachidonate release.

Incubation of the cells with a combination of milrinone plus rolipram or Ro 20-1724 elicited a significant reduction in fMLP-induced arachidonate release. db-cAMP, but not 8-br-cGMP, moderately but significantly inhibited arachidonate release (fig. 5).

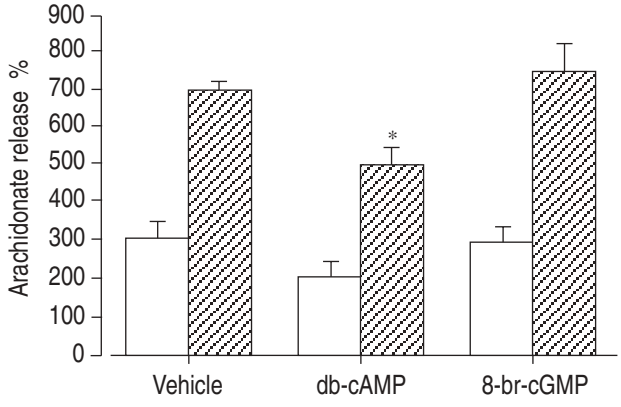

Fig. 5. – Effect of the analogue of cyclic adenosine monophosphate (cAMP) N^6-2'-*O*-dibutyryl cAMP (db-cAMP; 10^{-3} M), and the analogue of cyclic guanosine monophosphate (cGMP), 8-bromo cGMP (8-br-cGMP; 10^{-3} M), on arachidonate release by *N*-formyl-Met-Leu-Phe (fMLP)-stimulated alveolar macrophages collected from saline-exposed (▭) or ovalbumin (OA)-challenged (▨) sensitized guinea-pigs. Alveolar macrophages were collected 48 h following challenge and incubated *in vitro* for 30 min with the compounds. Results are expressed as means (percentage of release) of arachidonate±SEM from four experiments performed in triplicate. *: p<0.05 compared with vehicle.

Discussion

Airway hyperresponsiveness induced by OA challenge is generally associated in humans and in experimental animals with an influx of inflammatory cells in lung tissue [14, 17] and the activation of resident pulmonary cells such as alveolar macrophages [13, 18, 19]. The present study reports that macrophages recovered in the BAL fluid of either saline-exposed or OA-challenged sensitized guinea- pigs exhibited PDE types 3 and 4 isoenzyme activity. This was clearly observed by the inhibitory activities of the selective PDE4 inhibitor rolipram and the selective PDE3 inhibitor SKF 94836. Therefore, it is suggested that the profile of PDE activities in the macrophage may influence the functional control by PDE inhibitors. This result is consistent with previous studies performed on human ma-crophages, reporting the presence of PDE3 and PDE4 isoenzymes [20, 21]. This may be compared with the presence of PDE4 alone in monocytes [21, 22] or in a human monocytic cell line [23]. It has also been reported that the *in vitro* differentiation of human peripheral blood monocytes into macrophages is characterized by an increase in PDE3 activity, resulting in a PDE isoenzyme profile characteristic of mature macrophages [8, 21].

Increased PDE4 activity in peripheral blood monocytes from patients with atopic dermatitis has been reported [24]. If PDE4 induction occurs in inflammatory cells in asthma, possibly in response to pro-inflammatory cytokines [25], this would result in reduced intracellular cAMP levels, leading to increased activity of inflammatory cells [26–28]. In the present experimental conditions, there were no significant differences in PDE isoenzyme profiles when comparing alveolar macrophages from either saline-exposed or OA-challenged sensitized guinea-pigs. This is consistent with the results obtained by TENOR *et al.* [21], who reported no difference between alveolar macrophages from normal donors and from atopic asthmatics. Whether the enzyme(s) that are elevated following antigen challenge in sensitized guinea-pigs or in atopic patients are activated or subject to differential inhibition by PDE inhibitors is unknown. For this reason, the level of intracellular cAMP in alveolar macrophages from either saline-exposed or OA-challenged guinea-pigs was investigated.

The present results show that OA challenge induced a sustained increase in intracellular cAMP at 48 h. This enhanced level of intracellular cAMP was seen following incubation of the cells with the nonselective PDE inhibitor IBMX. In contrast, no change was observed at 1 h. These results are consistent with those reported by BEUSENBERG *et al.* [29], showing an increase in intracellular cAMP level, in alveolar macrophages after OA challenge in sensitized guinea-pigs. The observed rise in cAMP levels dur-ing OA challenge seems to be in contradiction to an expected increase in macrophage activity, since increases in intracellular cAMP levels are generally reflected by a decrease in cellular activity. However, the interaction of pro-inflammatory mediators, released during OA challenge, such as PGE_2, with the adenylyl cyclase system results in a rise in intracellular cAMP levels. Therefore, it is conceivable that a transient and rapid decrease in cAMP levels, allowing the release of mediators, may be followed by a marked and long-lasting enhancement, as observed in the present study.

OA challenge in sensitized guinea-pigs elicits an enhanced activity of alveolar macrophages, as demonstrated by the increased release of superoxide anions [13] or arachidonate after *in vitro* stimulation by fMLP (present study). The release of arachidonate was significantly reduced upon oral treatment of the guinea-pigs by the selective PDE4 inhibitors rolipram and Ro 20-1724, but not by the selective PDE3 inhibitor milrinone. In agreement with a previous study on the reduced activity of guinea-pig alveolar macrophages by PDE4 inhibitors [30], the present data confirm the potential interest of selective PDE4 inhibitors as anti-inflammatory drugs. It was previously demonstrated that, at the same doses, rolipram and Ro 20-1724 significantly reduced the recruitment of eosinophils in guinea-pigs induced by OA challenge, platelet-activating factor or interleukin-5 [16, 31]. However, the relationship between the recruitment of granulocytes in BAL and the reactivity of alveolar macrophages is not clear. It might be expected that the presence of granulocytes and cell fragments in the BAL fluid may activate the phagocytic activity of macrophages and, therefore, their ability to release various mediators.

When the alveolar macrophages were collected in the BAL fluid 24 h after OA challenge, Ro 20-1724 but not rolipram had a significant inhibitory effect, whereas incubation of the cells with the PDE3 inhibitor milrinone did not reduce arachidonate release. These results are not consistent with the previous data obtained on mononuclear cells from healthy subjects [6]. It is not clear why rolipram is ineffective in inhibiting arachidonate release. Indeed, rolipram and Ro 20-1724 elicited a marked and significant inhibition of arachidonate release from mononuclear cells [6] at lower concentrations (10^{-7} M and 10^{-6} M, respectively) than were used in the present study. Therefore, it is possible that alveolar macrophages are less sensitive than peripheral blood mononuclear cells to PDE4 inhibitors. This sensitivity could be due to the difference in the membrane permeability and/or in the cell's isoenzyme content. Indeed, it is well recognized that monocytes only contain PDE4 isoenzymes, whereas macrophages contain both PDE3 and PDE4 isoenzymes (this study and [8, 21]). This would strongly influence the reactivity of the cells according to their phenotypes. In this regard, the combination of milrinone plus the selective PDE4 inhibitors rolipram or Ro 20-1724 also had a significant inhibitory effect. This suggests that PDE4 plays a role in the control of mediator release from alveolar macrophages and that this control is enhanced when the PDE3 isoenzyme is inhibited.

Finally, in common with previous results in mononuclear cells [18], the cell-permeable analogue of cAMP, db-cAMP, elicited a significant reduction in arachidonate release from macrophages recovered in the BAL fluid of guinea-pigs after OA challenge, whereas the cGMP analogue was ineffective. This represents direct evidence that a selective increase in intracellular cAMP (but not cGMP) reduces arachidonate release. This also suggests that PDE4 and PDE3 inhibitors, which block the breakdown of cAMP by PDE, reduce the arachidonate release, in part, through the cAMP–protein kinase A pathway.

In conclusion, the present data show that the phosphodiesterase-3 and -4 isoenzymes are present in alveolar macrophages from sensitized guinea-pigs and that phosphodiesterase-4, and to a lesser extent, phosphodiesterase-3,

regulate the release of inflammatory mediators such as arachidonic acid through an increase in intracellular cyclic adenosine monophosphate levels. However, it seems that the inhibitory activity of phosphodiesterase-4 inhibitors *in vivo* occurs by interaction with others cells rather than a direct effect on alveolar macrophages. These results also support the potential utility of selective phosphodiesterase inhibitors in the treatment of asthma and other inflammatory disorders of the airways.

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