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Stimulation of cyclic AMP production in human alveolar macrophages induced by inflammatory mediators and β -sympathomimetics

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We have investigated the effects of inflammatory mediators and β -adrenoceptor agonists on the adenylyl cyclase responsiveness in alveolar macrophages from control subjects, patients suffering from chronic obstructive pulmonary disease (COPD) and asthmatics. Basal cyclic AMP (cAMP) levels in alveolar macrophages from COPD patients were significantly elevated (plus 42%) as compared to controls. In addition, the adenylyl cyclase responsiveness to prostaglandin E₂, histamine and the β -adrenoceptor agonist salbutamol was significantly impaired in alveolar macrophages from COPD patients and asthmatics. The lipid mediator platelet activating factor showed no effect on cAMP production in all three alveolar macrophage populations. Furthermore, the cAMP-enhancing effects of isoprenaline, salbutamol and histamine appeared to be mediated via β_2 -adrenoceptors and histamine H₂-receptor subtypes respectively. Taken together, these data suggest an intrinsic desensitization phenomenon in alveolar macrophages from COPD patients and asthmatics.

Alveolar macrophages; cAMP; Chronic obstructive pulmonary disease; Asthma; Adenylyl cyclase; Inflammatory mediators; β -Adrenoceptor agonists

1. Introduction

Pulmonary inflammation is incontrovertibly associated with chronic obstructive pulmonary disease (COPD) and asthma (Chung, 1986). Within the complex pathophysiological processes, alveolar macrophages exert a predominant role. Firstly, these cells act as scavengers in the first line of host defense by means of phagocytosis and related features like the production and release of reactive oxygen radicals and lysosomal enzymes (Fels and Cohn, 1986; Takemura and Werb, 1984). Secondly, alveolar macrophages retain a large potency to modulate the activity of other pulmonary cells via the release of inflammatory mediators like prostaglandins, leukotrienes and platelet activating factor (Morley et al., 1979; Arnoux et al., 1980; Fels et

al., 1982; MacDermot et al., 1984; Martin et al., 1984) and cytokines like interleukin-1 (IL-1) and tumor necrosis factor- α (TNF- α) (reviewed by Sibille and Reynolds, 1989; Kelley, 1990).

With respect to functional activity between alveolar macrophages from controls, COPD patients and asthmatics, several conflicting results have been reported. Thus, eicosanoid release from alveolar macrophages of asthmatics and COPD patients has been shown to be impaired (Godard et al., 1982), unaltered (Balter et al., 1988) or even enhanced (Damon et al., 1989; Chavis et al., 1991). Furthermore, the release of platelet activating factor (PAF), oxygen radicals and β -glucuronidase appears to be enhanced in alveolar macrophages from asthmatics (Tonnel et al., 1986; Arnoux et al., 1987; Cluzel et al., 1987).

Pulmonary mediators and other substances like hormones and drugs interact with cells via specific surface receptors coupled to transmembrane signalling systems which produce several second messengers (reviewed by Barnes, 1987). Functional activity of alveolar macrophages (and cells in general) is largely associated

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with intracellular levels of cyclic AMP (cAMP), a second messenger produced by the action of adenylyl cyclase. In general, high levels of cAMP coincide with down-regulation of functional activity (Bonta and Parnham, 1982).

Though the effects of inflammatory mediators on general aspects of pulmonary inflammation have been clearly described, reports on their effects on alveolar macrophages remain scarce. Histamine, leukotrienes and PAF have potent bronchoconstrictive actions, stimulate mucus secretion and exert strong chemotactic potency for inflammatory cells (Goetzl and Pickett, 1981; Hannah et al., 1981; Finney et al., 1985; Wardlaw et al., 1986; Persson, 1988). Prostaglandins of the E-type have mainly bronchodilating properties (Gardiner, 1975). To further investigate the role of mediators in pulmonary inflammation, we performed studies on the effects of histamine, prostaglandin E₂ (PGE₂), prostacyclin (PGI₂) and PAF on adenylyl cyclase responsiveness in human alveolar macrophages from controls, COPD patients and asthmatics. In addition, the effects of the β -adrenoceptor agonists isoprenaline and salbutamol (frequently used as anti-asthmatic drugs) on adenylyl cyclase responsiveness were determined as well.

2. Materials and methods

2.1. Subjects

Smoking female volunteers (> 5 pack years, age 23–37 years, mean age 30 years) were studied. None of the subjects had a history of pulmonary disorders or received any medication 2 months prior to the study. Informed consent for bronchoalveolar lavage (BAL) was obtained. BAL was performed under general anesthesia using a fiber optic bronchoscope. Four subsequent volumes of 50 ml sterile saline were instilled into a subsegmental bronchus of the right middle lobe, followed by gentle aspiration. The obtained BAL fluids were kept on ice until further isolation of alveolar macrophages.

2.2. Patients

The study included 12 COPD patients (seven female, five male, ages 24–75 years, mean age 51 years) and two non-atopic asthmatics (female, ages 31 and 37 years). Diagnosis of COPD patients (chronic bronchitis, $n = 6$; bronchial emphysema, $n = 6$) was based on a history of clinical symptoms, chest X-ray and pulmonary function tests. Mean forced expiratory volume (FEV₁) was $72 \pm 6.9\%$ and mean forced expiratory vital

capacity (FVC) was $83 \pm 4.0\%$ (percentage of predicted value). All of them were tobacco smokers (> 13 pack years), three received β -sympathomimetics and one corticosteroids. Bronchial asthma was diagnosed on the basis of clinical history, severity of airway obstruction (FEV₁ less than 70% of predicted value) and partial reversibility after inhalation of β -adrenoceptor agonists.

2.3. Isolation of alveolar macrophages

BAL fluids were filtered through surgical gauze and centrifuged at $400 \times g$ (10 min, 4°C). If necessary, erythrocytes were lysed by hypo-osmotic shock. The pellet was resuspended in Gey balanced salt solution (GBSS), pH 7.4 and alveolar macrophages were purified by density gradient centrifugation ($400 \times g$, 30 min, 4°C) using Ficoll-Isopaque (Nycomed, Oslo, Norway). After extensive washing, more than 95% of the isolated cell suspension consisted of alveolar macrophages as judged by May-Grünwald-Giemsa staining. Viability of the cells was assessed by dye exclusion using Trypan blue and alveolar macrophage suspensions with a viability exceeding 95% were used for the experiments.

2.4. Incubation procedure

1 ml samples of alveolar macrophages (10^6) were incubated in GBSS at 37°C in the presence of $400 \mu\text{M}$ IBMX (3-isobutyl-1-methylxanthine, Janssen Chimica, Beerse, Belgium) with histamine (Pharmacy Department, Dijkzigt Hospital, Rotterdam, Netherlands), prostaglandin E₂ (PGE₂), prostacyclin (PGI₂), platelet activating factor (PAF), salbutamol or isoprenaline (all from Sigma, St. Louis, MO, USA) dissolved in GBSS buffer. In some experiments cell suspensions were preincubated for 10 min with propranolol (Ciba-Geigy, Basel, Switzerland), cimetidine (Sigma, St. Louis, MO, USA) or mepyramine (Rhône-Poulenc, Paris, France). Following a 15 min incubation, cells were spun down and resuspended in $150 \mu\text{l}$ Tris-HCl buffer (pH 7.4) and boiled for 3 min. Cellular content of cAMP was determined by radioimmunoassay using [³H]cAMP (Amersham, Amersham, UK) and a high-affinity binding protein as described previously (Bonta et al., 1984).

2.5. Statistical analysis

Data are expressed as means \pm S.E.M. Statistical significance was evaluated by the unpaired Mann-Whitney U test. A P value of < 0.05 was considered significant.

3. Results

Table 1 shows the cellular differentiation of the BAL fluids from the three different groups of subjects (controls, COPD patients and asthmatics). Alveolar macrophages make up the majority of the recovered cells (91.5–95.5%) with a marked decrease in the percentage in the BAL fluids from COPD patients and an increase in the number of lymphocytes, eosinophils and neutrophils.

Basal cAMP levels were significantly higher (plus 42%) in alveolar macrophages from COPD patients as compared to control alveolar macrophages, while alveolar macrophages from (two) asthmatics showed the same level as controls (cf. table 2).

The stimulatory effects of inflammatory mediators (PGE_2 , PGI_2 , PAF and histamine) and β -sympathomimetics (isoprenaline and salbutamol) on cyclic AMP production in different alveolar macrophage populations are depicted in figs. 1 and 2. Prostaglandin E_2 and histamine dose-dependently stimulated cAMP

TABLE 1

Cellular differentiation (in percentages) of BAL fluids from controls, COPD patients and asthmatics.

Number of observations in parentheses.

	Controls (22)	COPD patients (12)	Asthmatics (2)
Macrophages	95.5 ± 0.6	87.2 ± 3.6 *	91.5 ± 0.5
Lymphocytes	2.3 ± 0.4	4.3 ± 0.6 *	3.5 ± 2.5
Eosinophils	0.6 ± 0.2	1.9 ± 0.7 *	3.2 ± 2.5
Neutrophils	0.3 ± 0.1	4.8 ± 2.8 *	0.4 ± 0.9
Mononuclear cells ^a	1.3 ± 0.8	1.8 ± 0.6	1.4 ± 0.7

^a Other than macrophages or lymphocytes.

* $P < 0.05$ as compared to controls.

production in the three alveolar macrophage populations, though less effectively in alveolar macrophages from COPD patients and asthmatics (cf. figs. 1a and 1d respectively). Using PGI_2 , no difference in adenylyl cyclase responsiveness among the three different alveolar macrophage populations (cf. fig. 1b) was observed.

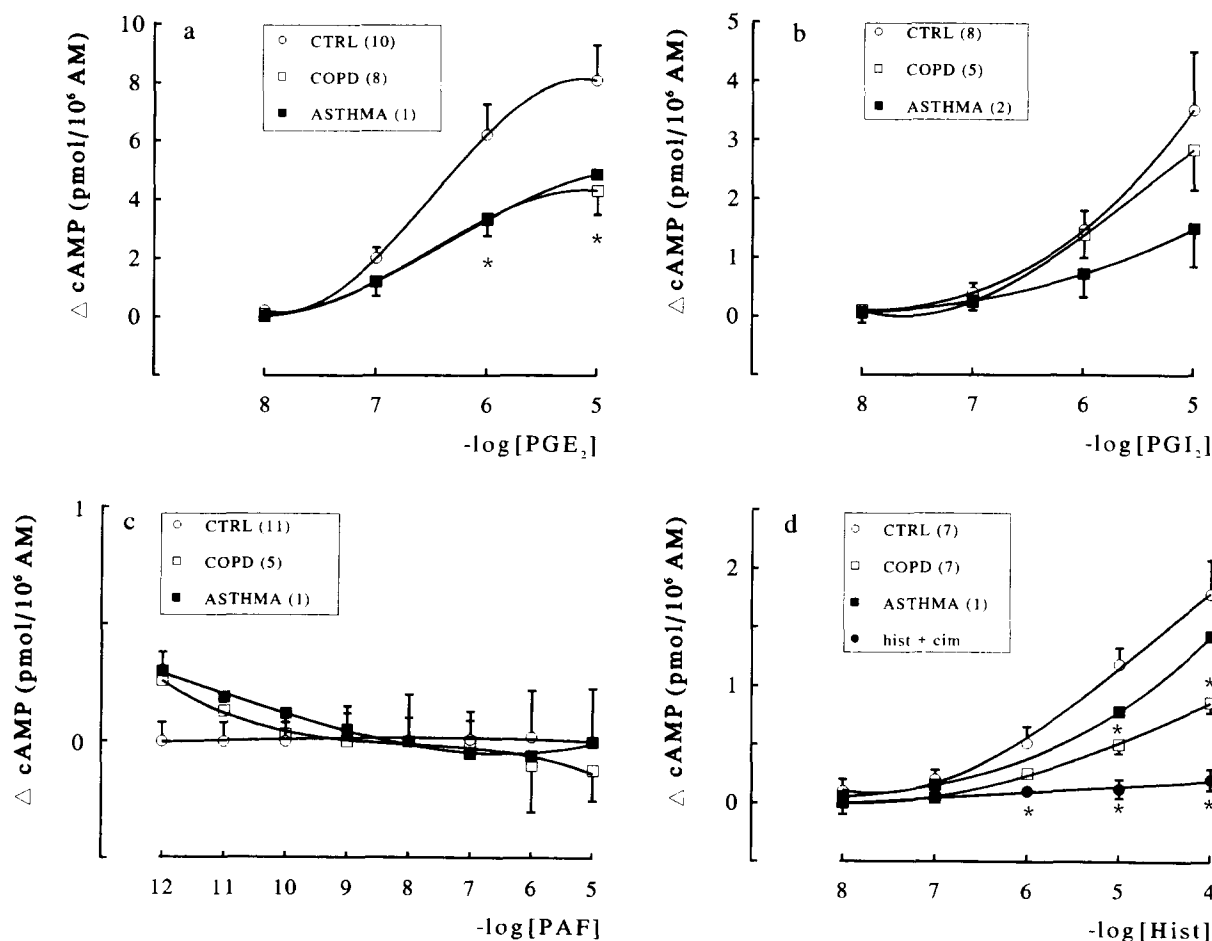


Fig. 1. Cyclic AMP production in alveolar macrophages (AM) from control subjects (CTRL, open circles), COPD patients (COPD, open squares) and asthmatics (ASTHMA, closed squares) following a 15 min incubation with the inflammatory mediators PGE_2 (a), PGI_2 (b), PAF (c), and histamine (d) in the presence of $400 \mu\text{M}$ IBMX. Prior to incubation with histamine, control alveolar macrophages were incubated with the H_2 -selective antagonist cimetidine 10^{-5} M (panel d, closed circles). Number of duplicate observations in parentheses. Data are expressed as mean absolute increase above basal cAMP level \pm S.E.M. ($\text{pmol}/10^6$ alveolar macrophages). * $P < 0.05$ as compared to controls.

TABLE 2

Basal cAMP levels in alveolar macrophages from controls, COPD patients and asthmatics.

Data are expressed as mean \pm S.E.M. (pmol/10⁶ alveolar macrophages) from 22 (controls), 12 (COPD patients) and two (asthmatics) duplicate experiments.

	cAMP (pmol/10 ⁶ alveolar macrophages)
Controls	1.63 \pm 0.11
COPD patients	2.31 \pm 0.28 *
Asthmatics	1.57 \pm 0.93

* P < 0.05 as compared to controls.

The lipid mediator PAF does not affect cAMP levels in alveolar macrophages (cf. fig. 1c). At the lowest concentration (10⁻¹² M), however, PAF tends to enhance cAMP production in alveolar macrophages from COPD patients (+15%) and asthmatics (+17%).

The adenylyl cyclase responsiveness to isoprenaline and salbutamol shows similar results as the response of the inflammatory mediators. The non-selective β -adrenoceptor agonist isoprenaline enhances cAMP levels in all three alveolar macrophage populations with the same potency (cf. fig. 2a), whereas the response to the β_2 -selective adrenoceptor agonist salbutamol is largely reduced in alveolar macrophages from COPD patients and asthmatics (cf. fig. 2b).

The stimulatory effects of isoprenaline and salbutamol on cAMP production were completely blocked by propranolol 10⁻⁵ M (cf. figs. 2a and 2b). Considering the high potency of salbutamol, β_2 -adrenoceptors appear to mediate this action. The histaminergic effect is attained by stimulation of histamine H₂ receptors as the H₂-selective antagonist cimetidine (10⁻⁵ M) completely reversed the effect (cf. fig. 1d) while the H₁-

selective antagonist mepyramine (10⁻⁵ M) was in this respect not effective (results not shown).

It should be mentioned that due to the poor availability of alveolar macrophages from asthmatics, we were not able to determine the adenylyl cyclase responsiveness more adequately in this alveolar macrophage population.

4. Discussion

Recently, we have shown in human alveolar macrophages that the β_2 -adrenergic agonist salbutamol and the phosphodiesterase inhibitor IBMX inhibited PGE₂ release and stimulated leukotriene B₄ (LTB₄) secretion via enhancement of intracellular cAMP levels (Beusenbergh et al., submitted for publication). Hence, we were interested to determine whether inflammatory mediators and β sympathicomimetics showed the same potency to stimulate cAMP production in alveolar macrophages from controls, COPD patients and asthmatics.

Cellular composition of BAL fluids from the three groups of subjects differed largely with respect to the number of alveolar macrophages, eosinophils, neutrophils and lymphocytes which is in accordance with previous results. Thus, an increase in the number of predominantly eosinophils in BAL fluids from asthmatics has been reported by Wardlaw et al. (1988) and Tomioka et al. (1984) whereas in BAL fluids from COPD patients an increase of neutrophils has been reported (Martin et al., 1985). The increase in the number of lymphocytes in COPD patients and asthmatics (both groups consisting of non-allergic subjects)

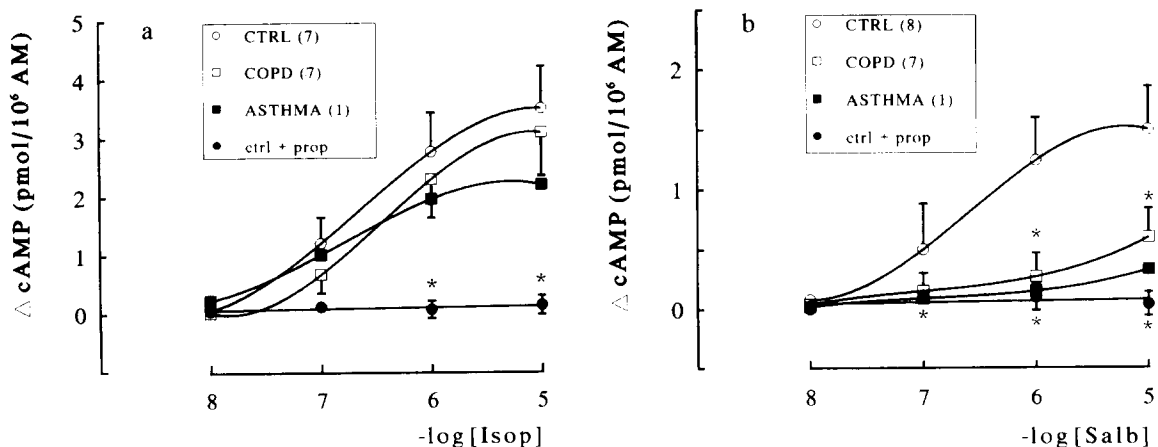


Fig. 2. Cyclic AMP production in alveolar macrophages (AM) from control subjects (CTRL, open circles), COPD patients (COPD, open squares) and asthmatics (ASTHMA, closed squares) following a 15 min incubation with the β -adrenoceptor agonists isoprenaline (Isop, panel a) and salbutamol (Salb, panel b) in the presence of 400 μ M IBMX. Prior to incubation with the agonists, control alveolar macrophages were incubated with the non-selective β -adrenoceptor antagonist propranolol (prop, 10⁻⁵ M, closed circles). Number of duplicate observations in parentheses. Data are expressed as mean absolute increase above basal cAMP level \pm S.E.M. (pmol/10⁶ alveolar macrophages). * P < 0.05 as compared to controls.

observed by us and others (Gonzalez et al., 1987; Metzger et al., 1987) suggests that these cells, besides alveolar macrophages, eosinophils and neutrophils, may play an important role in pulmonary inflammation.

The present results show that initial basal cAMP levels differed largely between the alveolar macrophage populations. Alveolar macrophages from COPD patients contained some 40% more cAMP than control alveolar macrophages whereas basal cAMP levels in alveolar macrophages from asthmatics showed no differences compared to controls.

For different reasons, it is difficult to interpret our data on basal cAMP in alveolar macrophages from asthmatics with functional parameters as conflicting data have been reported. Thus, while some reports indicate altered functional activity (e.g. chemiluminescence and arachidonic acid metabolism) in alveolar macrophages from asthmatics (Godard et al., 1982; Cluzel et al., 1987; Damon et al., 1989), others have reported similar results between alveolar macrophages from asthmatics and controls (Balter et al., 1988). In addition, our results were merely based on two asthmatics (which suggested no differences in basal cAMP levels). Whether functional activity of alveolar macrophages from asthmatics indeed differs from controls remains to be established.

One can only speculate about the origin of the enhanced basal cAMP levels in alveolar macrophages from COPD patients. Possibly, the persistent local inflammatory environment generates various mediators, like PGE₂, PGI₂ and histamine which stimulate cAMP production in alveolar macrophages located in the alveolar compartment. Like in other tissues and cells (Remold-O'Donnell, 1974; Meurs et al., 1985), such continuous exposure of alveolar macrophages to inflammatory mediators, which may stimulate adenylyl cyclase, will ultimately induce a desensitization of the stimulatory receptors. Consequently, alveolar macrophages will become less susceptible to respond to these inflammatory substances. The present data on the diminished responsiveness of the adenylyl cyclase-coupled signal transduction system to various mediators and drugs suggest a heterologous desensitization phenomenon. The differences in potency between the β -adrenoceptor agonists isoprenaline and salbutamol to stimulate adenylyl cyclase is in accordance with previous results. Using guinea pig alveolar macrophages, we suggested that the observed differences are probably due to the partial agonistic effect of salbutamol (Beusenberg et al., 1989).

The few data on alveolar macrophages from asthmatics presented here point to a similar desensitization of the adenylyl cyclase system in alveolar macrophages, suggesting that asthma shares some common immunoregulatory- and inflammatory-related mechanisms with COPD.

Within the pathophysiology of asthma and COPD, data on the phenomenon of desensitization in pulmonary cells are limited and have been confined to mainly bronchial smooth muscles and blood leukocytes. It is suggested that diminished β -adrenoceptor function in asthmatics is probably a consequence of the active disease state (following allergen challenge) rather than an intrinsic component of asthma (reviewed by Nijkamp and Henricks, 1990). In the present report, however, we present evidence that a general (including β -adrenoceptor) dysfunction of the adenylyl cyclase system in alveolar macrophages, an important cellular component within the pulmonary compartment, is a general and intrinsic feature of pulmonary inflammation associated with asthma and COPD.

Physiologically, the impaired responsiveness of the adenylyl cyclase system to stimulatory agents in alveolar macrophages from COPD patients and asthmatics would implicate that cellular functions of the alveolar macrophages which are affected by alterations in cAMP levels (like oxygen radical and enzyme production) are less susceptible to modulation by external factors. Whether this renders the alveolar macrophages more sensitive to external modulation via mechanisms distinct from the adenylyl cyclase pathway remains to be investigated.

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