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CYCLIC AMP ENHANCING DRUGS MODULATE EICOSANOID RELEASE FROM HUMAN ALVEOLAR MACROPHAGES

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Summary

The effect of the phosphodiesterase inhibitor isobutyl-methylxanthine (IBMX), salbutamol and sodium nitroprusside was evaluated regarding PGE_2 and LTB_4 release and cAMP and cGMP level in human alveolar macrophages obtained from controls and COPD patients. Basal levels per five million control- respectively COPD alveolar macrophages: cAMP 1.2 and 1.0 pmole; cGMP 8.4 and 9.1 fmole; PGE_2 120 and 63 pg and LTB_4 19.2 and 14.8 pg. In both populations IBMX increased cAMP level by 55-93% and salbutamol+IBMX by 285-252%. Except for the 61% rise in LTB_4 release by salbutamol+IBMX the drugs hardly affected PGE_2 and LTB_4 release from control macrophages. In COPD alveolar macrophages, however, IBMX and IBMX+salbutamol largely reduced PGE_2 release (63 vs 11 pg per 10^6 cells) but less efficiently increased LTB_4. In both macrophage populations sodium nitroprusside (SNP) substantially increased (3-4 fold) cGMP level but did not affect eicosanoid production. Present results indicate that drugs which enhance cAMP level decrease PGE_2 release from COPD macrophages and stimulate the release of LTB_4 a chemotactic mediator involved in bronchial inflammatory reactions.

Key Words: alveolar macrophages, cyclic AMP, eicosanoid release

Airway inflammation is a characteristic feature of both asthma and chronic obstructive pulmonary diseases (COPD) resulting from largely unknown pathophysiological events (1). Alveolar macrophages (AM) modulate the activity of other cells via the release of cytokines, prostaglandins, leukotrienes and PAF (2,3). The released eicosanoids, notably LTB_4 (4-5) and PGE_2 (6) may affect via feedback mechanism(s) macrophage activity itself (7-9) or the activity of other cells (10). LTB_4 for instance is chemotactic for mast cells (11), eosinophils (12) and

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neutrophils (13). Stimulated AM from asthmatic patients release larger amounts of inflammatory mediators than AM derived from healthy subjects (14,15) though others did not confirm this (16). Release of lipid mediators (prostaglandins, leukotrienes and PAF) from AM may have important implications for the micro-environment of the bronchoalveolar compartment.

The modulatory effects of AM depend on various factors like basal intracellular cAMP level, state of priming and exposure to biologically active substances. Increase in intracellular cAMP level generally decreases cellular activity. For instance elevation of cAMP inhibits zymosan-stimulated arachidonic acid release in monocytes (17) and the production of thromboxane B_2 and LTB_4 in elicited peritoneal macrophages (18). Whether in addition cGMP level is also of importance in the control of cellular activity is not well established. Zymosan elicits a rapid rise in both cAMP and cGMP formation (19) and muramyl dipeptide (20), sodium nitroprusside (19), and exogenous cGMP have been shown to enhance macrophage activity (20,21). Others, however, do not observe macrophage activation by inducers of cGMP production (19).

The effect of bronchodilating drugs (salbutamol, sodium nitroprusside and theophylline-like compounds) are believed to be beneficial only. However, by changing intracellular cyclic nucleotide levels these drugs may in addition alter the functional activity of AM, known to be involved in airway inflammation.

Materials and Methods

Ten patients (female, age 25-40 yrs, mean age 31, all smokers, > 7 pack years) hospitalised for obstetric purposes were studied. None of these subjects had a history of pulmonary disorders or received any medication two months prior to the study. Informed consent for bronchoalveolar lavage (BAL) was obtained. Alveolar cells were obtained by BAL under general anaesthesia using a fiberoptic bronchoscope. BAL fluids containing a total of 25-85 $\cdot 10^6$ cells were subsequently kept on ice.

Lung tissue was obtained from 8 smoking (> 10 pack years) COPD patients with peripheral carcinoma (6 male, 2 female, mean age 51 years) who had undergone thoracotomy. According to the criteria of the American Thoracic Society all patients were diagnosed for COPD. Patients receiving beta-sympathomimetics or theophylline were excluded from the study. Mean FEV_1 was 65.7%, mean FVC was 87.3% (calculated from normal predicted value). Within 30 min after surgical resection tissue was immersed in ice-cold buffer. AM were recovered by *in-vitro* lavage of segments using a 20 ml syringe.

BAL fluids were filtered through surgical gauze and centrifuged at 400g (10 min, 4°C). The pellet was resuspended in Gey Balanced Salt Solution (GBSS), pH 7.4 and AM were purified by Ficoll-Isopaque centrifugation (400g, 30 min, 4°C) and the resultant AM layer was washed twice. May Grünwald Giemsa staining showed that the suspension contained approximately 96% (control) and 86% (COPD) AM. Viability of the cells was at least 95%. Cells were not further purified via culturing to avoid frustrated phagocytosis eliciting cell activation.

AM ($2 \cdot 10^6$ ml^{-1}) were incubated in GBSS buffer at 37°C for 60 min and drugs dissolved in GBSS. In some experiments cell suspensions were pre-incubated for 15 min with 10 μM propranolol. Incubation was stopped by centrifugation (1 min, 15,000g) and the supernatant was removed, freeze dried and stored at -80°C for analysis of eicosanoids. The pellet was resuspended in 150 μl Tris-HCl buffer (pH 7.4) and boiled for 3 min. PGE_2 and LTB_4 were assayed by ELISA. Detection limits for PGE_2 and LTB_4 were 3 and 1 pg/ml. Cellular cyclic

AMP was determined by radioimmunoassay using [^{3}H] cAMP and an isolated binding protein (22). Cellular cGMP levels were determined by radioimmunoassay after acetylation (23).

Ficoll-Isopaque (Nycomed, Oslo, Norway); IBMX (isobutyl-methylxanthine, Janssen Chimica, Beerse, Belgium); salbutamol, sodium nitroprusside (SNP) and BSA (Sigma, St-Louis, USA); propranolol (Ciba-Geigy, Basel, Switzerland); ELISA kits for PGE_2 and LTB_4 (Cayman Chemical, Ann Arbor, USA); [^{3}H] cAMP and [^{125}I] succinyl-cGMP tyrosine methyl ester (Amersham International, Amersham, UK).

Data are expressed as means ± s.e.mean. Statistical significance was evaluated by the Mann-Whitney U test. A P value < 0.05 was considered significant.

Results

Cellular composition of BAL and basal values of control macrophages

Despite the use of different techniques to harvest broncholaveolar cells, cellular composition may be compared (24). Analysis of the cellular composition of BAL fluids showed large differences between controls and COPD subjects. BAL fluids of controls contained mainly AM (95%) and some lymphocytes (3.6%). In BAL fluids obtained from COPD patients considerable numbers of eosinophils (7.4%), neutrophils (14.4%) and lymphocytes (10.6%) were present.

Basal values and effects of drugs on cyclic nucleotide levels and eicosanoid release in control AM are denoted in Table 1. In controls, basal cAMP and cGMP level per 10^6 AM was 1.4 pmole and 8.4 fmole, reflecting a 160-fold difference in concentration.

Levels of cyclic nucleotides and eicosanoid release in control macrophages

In the presence of the nonselective phosphodiesterase inhibitor IBMX cAMP and cGMP levels rose to respectively 193% and 155% of basal level (cf. Table 1). Eicosanoid release from AM was only slightly though significantly affected by IBMX: per 10^6 AM PGE_2 release decreased from 120 pg to 90 pg and LTB_4 release increased from 19 to 24 pg.

TABLE 1

Cyclic nucleotide levels and eicosanoid release in control human AM. Data are expressed as mean ± s.e.mean from 8-10 duplicate experiments. Salb.: salbutamol; SNP: sodium nitroprusside.

	pmole cAMP/10^6AM	fmole cGMP/10^6AM	pg PGE_2/10^6AM	pg LTB_4/10^6AM
saline	1.4±0.1	8.4±1.1	120±5	19.2±4.2
1 mM IBMX	2.7±0.3*	13.0±1.7*	90±6*	24.2±3.5*
1 mM IBMX + 10 μM salb.	4.0±0.4**	13.3±2.3	80±3	30.4±3.1**
1 mM IBMX + 1 mM SNP	2.2±0.3^{NS}	24.3±2.9**	98±6	25.4±5.1

* P<0.05 vs saline; ** P<0.05 vs IBMX alone; ^{NS} non-significant vs IBMX alone.

TABLE 2

Cyclic nucleotide levels and eicosanoid release in AM from COPD patients. Data are expressed as mean ± s.e.mean from 7-8 duplicate experiments. Salb.: salbutamol. SNP: sodium nitroprusside.

	pmole cAMP/10^6AM	fmole cGMP/10^6AM	pg PGE_2/10^6AM	pg LTB_4/10^6AM
saline	1.0±0.1†	9.1±1.3	63±9	14.8±0.5
1 mM IBMX	1.5±0.3*	16.0±1.9*	11±6*	18.3±2.0*
1 mM IBMX + 10 μM salb.	2.5±0.3**	13.6±2.2	9±3	16.2±0.3
1 mM IBMX + 1 mM SNP	1.5±0.2	37.4±2.4**,§	6±3	17.2±0.8

† P<0.05 compared to control AM; * P<0.05 vs saline; ** P<0.05 vs IBMX alone; § difference in effect of SNP between control-AM and COPD-AM is not significant.

The combination of IBMX with the selective $beta_2$-sympathomimetic salbutamol enhanced intracellular cAMP level more efficiently than IBMX alone (185% increase), but compared to IBMX PGE_2 release was hardly further inhibited. Considering LTB_4 release, however, salbutamol+IBMX was some two-fold more effective than IBMX alone (30.4 vs 24.2). Propranolol (10 μM) completely blocked the salbutamol effects (not shown). Sodium nitroprusside (SNP) substantially increases cellular cGMP concentration (additional 134%) but does not affect cAMP content or eicosanoid release.

Levels of cyclic nucleotides and eicosanoid release in AM from COPD patients

Table 2 shows that untreated AM from COPD patients (refered as COPD-AM) contained less cAMP (-29%) and released considerable less PGE_2 and LTB_4 compared to control AM (50% respectively 70%). The drugs IBMX and salbutamol enhanced cyclic nucleotide levels roughly to the same extent as in control AM. However, compared to control AM the reduction in PGE_2 release was much larger (83% vs 21%) while LTB_4 release was slightly enhanced (24%). Salbutamol induced in COPD-AM a further increase in cAMP level (additional +102% compared to IBMX) which was not related to additional changes in PGE_2- or LTB_4 release. SNP showed similar responses in COPD-AM and control AM.

Discussion

Considering basal values for cAMP and eicosanoid release some interesting differences between the macrophage populations of controls and COPD patients are observed. Others observe about 50-fold lower amounts of eicosanoid released which may be due to the purification technique used (adherence/culturing) enhancing 'basal' activation state due to frustated phagocytosis (25). In this process eicosanoid release rises, peaks, exhausts and returns to some resting level. Thus it is conceivable that subsequent exposure induces smaller amounts of released eicosanoids. Different results may also be obtained when the metabolism of a non-endogenous pool of tritiated arachidonic acid is determined.

The present study compares COPD-AM with control AM from patients with no history of

pulmonary disorder. Both groups of AM were obtained from smokers only to allow good comparison as it is generally known that smoking impairs certain AM functions including eicosanoid production (26). In contrast to control-AM, COPD-AM are recovered from patients with a pulmonary environment characterized by frequent unspecified inflammations (note the considerable number of inflammatory cells in the BAL of COPD patients). In addition, COPD-AM are obtained from a rather heterogeneous group of COPD patients consuming a variety of drugs. So COPD-AM differ predominantly from control-AM by their origine from an inflammatory environment which probably renders COPD-AM more elicited than control-AM.

Compared to control-AM, COPD-AM show significantly lower basal level in cAMP and PGE_2- and LTB_4 release and behave quite differently regarding induced eicosanoid release. Peritoneal macrophages obtained during peritoneal inflammation likewise showed lower basal cAMP level and PGE_2 release compared to macrophages collected from patients without such complications (27). Drug-induced increases in cellular cAMP level was related to decreased PGE_2 and a slight increase in LTB_4 release. These results are only partly comparable with previous results obtained in activated (elicited, cultured or primed) macrophages showing that elevation of cAMP level inhibited both PGE_2 and LTB_4 release (e.g. 18).

Interestingly, IBMX induces in the two AM populations a similar increase in cAMP level but affects PGE_2 release in COPD-AM to a much larger extent compared to control AM (decrease respectively 83 and 17%). Both macrophage populations show, however, a comparable increase in LTB_4 release in response to IBMX.

We suggest that in control AM the reduction of free arachidonic acid metabolism to PGE_2 results in a higher availability of the common substrate (free arachidonic acid) for leukotriene synthesis by 5-lipoxygenase. Simultaneous down-regulation of cyclooxygenase and enhanced 5-lipoxygenase activity has recently been observed by Mackenzie et al. (25) in macrophages exposed to calcium ionophore. The suggested inhibition of phospholipase A_2 and release of arachidonic acid from phospholipids by elevated cAMP (28) may only explain the decrease in PGE_2 release but not the change in LTB_4 release.

In both control AM and COPD-AM enhancement of cGMP level is not related to either PGE_2 or LTB_4 release indicating that the cytosolic pool of cGMP is not involved in the regulation of eicosanoid release from AM.

Due to their bronchodilating proporties the xanthine derivative theophylline and $beta_2$-sympathomimetics are frequently used in the treatment of asthma and COPD. Present results indicate that these drugs in addition modulate eicosanoid release from AM which may have important implications for the micro-environment in bronchoalveolar compartment.

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