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Prostaglandin E_2 in the expired breath condensate of patients with asthma

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Prostaglandin E_2 in the expired breath condensate of patients with asthma. K. Kostikas, G. Papatheodorou, K. Psathakis, P. Panagou, S. Loukides. ©ERS Journals Ltd 2003.
ABSTRACT: Inhaled prostaglandin (PG)E_2 has been found to cause bronchodilation in asthmatics, although it does not have bronchodilative effects in normal subjects. The aim of this study was to investigate the levels of PGE_2 in the expired breath condensate of patients with asthma, the possible contribution of smoking habit to its levels and the possible relationship between PGE_2 and the degree of bronchial hyperresponsiveness, as assessed by the provocation dose of histamine causing a 20% fall in forced expiratory volume in one second (FEV1) (PD20).

A total of 30 mild asthmatics (15 smokers, all steroid-naive, FEV1 88±6 (%±SD)) and 20 healthy control subjects (10 smokers) were studied. Histamine challenge testing was performed in all subjects and the PD20 was determined.

The results showed that asthmatic smokers had significantly higher values of PGE_2 compared to asthmatic nonsmokers and control subjects (40±21 *versus* 14.5±4.5 *versus* 11.7±3 pg·mL^{-1}, respectively). Further analysis showed that PGE_2 levels were significantly higher in asthmatic smokers compared to smoker and nonsmoker controls (40±21 *versus* 11.6±2 *versus* 11.7±4 pg·mL^{-1}, respectively). No significant difference was observed between asthmatic nonsmokers and both control smokers and control nonsmokers. No significant correlation was found between PGE_2 levels and PD20 in all groups of asthmatics, irrespective of smoking habit.

In conclusion, the elevation of prostaglandin E_2 in the expired breath condensate of patients with asthma is mainly attributed to smoking habit and prostaglandin E_2 levels do not predict the degree of bronchial hyperresponsiveness.
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Eicosanoids are important inflammatory mediators in asthma. These lipid mediators include leukotrienes, prostaglandins and thromboxanes. Prostaglandin (PG)E_2 is a dominant cyclooxygenase product of airway epithelium and smooth muscle, and is considered to be immunomodulatory and predominantly bronchoprotective [1].

PGE_2 inhibits both exercise-induced bronchoconstriction [2] and allergen-induced early and late asthmatic responses [3], and also prevents aspirin-induced bronchoconstriction in aspirin-sensitive asthma [4]. PGE_2 has also been shown to decrease exhaled nitric oxide [5] and to prevent the induction of inducible NO synthase in certain cell lines [6]. *In vitro* studies have shown that PGE_2 inhibits many inflammatory events, including mast cell mediator release and eosinophil activation [7].

The measurement of markers in the expired breath condensate (EBC) has proven to be a useful noninvasive method for the assessment and monitoring of airway inflammation [8]. EBC is a simple, noninvasive technique for monitoring airway inflammation. It reflects abnormalities in markers obtained bronchoscopically, in sputum and in exhaled air [8]. Measurement of PGE_2 in EBC of asthmatic patients, when compared to normal subjects, has not shown any significant differences [9, 10]. However, recent evidence suggests that cigarette smoke stimulates the formation of PGE_2 in airway macrophages [11].

Despite their clinical value, previous studies have not investigated the stability and repeatability of PGE_2 measurements at different time intervals, the effect of smoking on PGE_2 levels and the possible protective effect of high levels of PGE_2 in EBC during bronchial hyperresponsiveness induced by histamine challenge testing. The primary aim of the current study was to investigate the repeatability and stability of measurements, to evaluate PGE_2 concentration in the EBC of asthmatic patients, to account for the possible confounding effects of smoking habit and to see whether there is an association between PGE_2 concentration in EBC and bronchial hyperresponsiveness, as assessed by the provocative dose of histamine causing a 20% fall in forced expiratory volume in one second (FEV1) (PD20).

Methods

Subjects

The general characteristics of patients and controls are summarised in table 1. The recommendations of the Global Strategy for Asthma Management and Prevention [12] were used for the diagnosis and classification of asthma severity, in order to define the asthmatic patients. A total of 30 steroid-naive patients with mild asthma (15 current smokers (n±SD (range)) 13±5 (9–17) pack-yrs, FEV1 88±6% predicted) were studied. All patients were atopic, as judged by the positive skin-prick tests to six common aeroallergens and the elevated levels of total immunoglobulin (Ig)E (207±47 International

Table 1. – Subjects characteristics

	Controls			Asthma		
	All	Smokers	Nonsmokers	All	Smokers	Nonsmokers
Age	25±4 (18–32)	25±5 (18–32)	24.5±3 (19–29)	26±4 (19–34)	25±5 (19–33)	26±4 (19–34)
FEV1 % pred	95±7 (82–109)	94.5±7 (85–109)	96±8 (82–106)	88±6 (81–110)	89±7.5 (81–110)	87±3 (82–92)
PD20 mg	1.6±0.2 (1.45–1.85)	1.7±0.3 (1.45–1.85)	1.5±0.1 (1.47–1.82)	0.29±0.12 (0.15–0.63)	0.33±0.1 (0.17–0.62)	0.26±0.1 (0.15–0.63)
Smoking pack-yrs		14±4 (10–16)			13±5 (9–17)	

Data are presented as mean±SD (range). FEV1: forced expiratory volume in one second; PD20: provocation dose of histamine causing a 20% fall in FEV1.

Units (IU)·mL^{-1}). None of the patients was on any other anti-inflammatory treatment, including leukotriene antagonists, theophylline, and inhaled or oral mucolytics. Patients were clinically stable and asymptomatic at the time of the measurement and had no evidence of acute exacerbation. All measurements were performed out of allergy season and none of the patients had allergic symptoms or upper airway disease, either infectious or atopic.

A total of 20 control subjects were studied (10 current smokers with similar smoking habits as the asthmatic smokers, 14±4 (10–16) pack-yrs, FEV1 95±7% pred). All had a negative history of allergy (negative skin-prick tests to common allergens) and normal bronchial reactivity, with a PD20 of histamine >0.800 mg (1.6±0.2 (1.45–1.85)).

Medical history and smoking status were initially recorded in all study subjects. Subsequently, EBC collection was carried out. Finally, pulmonary function tests and histamine challenge were performed. This was mainly designed in order to prevent the bronchoconstriction occurring after histamine challenge having any effect on the PGE_2 values in the EBC-collected specimen.

The Ethics Committee of Athens Army General Hospital, Athens, Greece, approved the study protocol and all subjects gave informed written consent.

Lung function

FEV1 was measured with a dry spirometer (Vica-test, Mijnhardt, Holland). The best value of three manoeuvres was expressed as percentage of the predicted value. Bronchial hyperresponsiveness was measured by a rapid histamine inhalation test for the determination of PD20, as previously described [13]. PD20 was determined by linear interpolation on a semi-logarithmic scale. Asthmatic subjects stopped short- or long-acting adrenergic bronchodilators for ⩾12 h before the bronchial challenge. According to American Thoracic Society criteria [14], subjects were asked not to smoke 6 h before challenge.

Collection of expired breath condensate

The collection of EBC was performed as previously described [15]. Briefly, a heat exchanger unit (RHES, model 6V3; Jaeger, Wuerzburg, Germany) was used to produce cold air of -15°C–-18°C at an airflow of >80 L·min^{-1}. A double-jacketed glass tube of 45-cm length (internal diameter 4 cm and external diameter 7 cm) was specifically adapted to the cold air system and a two-way unidirectional valve (Series 2-200; Hans-Rudolf, Kansas City, MO, USA) was connected to the proximal end of the tube, in order to separate inspiration from expiration. After mouth rinsing, subjects were comfortably seated in a chair, wearing nose clips, and breathed in a relaxed manner (tidal breathing) for 15 min. The breath condensate was collected at the distal end of the tube and was immediately stored at -70°C for later analysis. According to this design, salivary contamination was highly unlikely and was easily observed, as the proximal cold air connection was 25 cm away from the mouthpiece. Approximately 2 mL of breath condensate was collected in a 2-mL sterile plastic tube.

In order to assess the repeatability of PGE_2 measurements and the stability of the frozen samples, 10 patients with mild asthma were evaluated before starting the whole protocol. All patients were asymptomatic and had similar characteristics as asthmatics participating in the study. Five of them were current smokers with similar smoking habits as the main study smoking population (13.5±3 (8–17) pack-yrs) and had normal lung function (FEV1 94±6% pred). All 10 patients received similar medical treatment to the main study population and were atopic, as judged by positive skin-prick tests to six common aeroallergens and elevated levels of total IgE (198±2 IU·mL^{-1}). The collection of EBC was performed under the same conditions used for the main study population.

Repeatability of PGE_2 measurements and stability of the frozen samples were estimated as previously described [16, 17]. Briefly, for repeatability data, EBC was collected on 2 consecutive days under the same conditions and a mean within-subject difference was measured. To assess the stability in the frozen condensate samples, 4 mL of condensate was collected using the same procedure under the same conditions but with a collection time of 20 min. The above-mentioned concentration was divided into 1 mL aliquots, which were each defrosted in the proportional time interval in which PGE_2 concentrations were determined, *i.e.* after 2 days, 1 week, 2 weeks and 3 weeks of storage (the maximum time between collection and measurement in the frozen samples). All condensate samples were tested for salivary contamination by determination of amylase activity, as previously described [15].

Prostaglandin E_2 measurement

PGE_2 concentrations in EBC were measured by a specific immunoassay kit (PGE_2-high sensitivity; R&D Systems Europe, Oxford, UK). The detection limit of the assay was 8.0 pg·mL^{-1}. Based on stability data, all condensate samples were measured within 2 weeks of the collection time.

Statistical analysis

Data concerning subject characteristics are expressed as mean±SD (range). Data concerning comparisons among the

PGE_2 values in the study groups are given as mean±SD. PGE_2 values from the study groups were compared using the one-way analysis of variance with an appropriate *post-hoc* test (Bonferroni) for multiple comparisons. Similar statistical analysis was used for statistical estimation of stability measurements. Repeatability data were assessed using the Bland and Altman′s test [18]. For normally distributed data (smoking and nonsmoking controls), the paired t-test was used for statistical comparisons between two groups. For non-normally distributed data (asthmatics and controls), the Mann-Whitney U-test was used for statistical comparisons between two groups. Normality of distribution was tested with Shapiro-Wilk′s test. Spearman′s correlation coefficient was used to investigate the relationships between parameters. A p-value <0.05 was considered significant.

Results

Repeatability data

The repeated measurements of PGE_2 in EBC on 2 consecutive days presented good repeatability. PGE_2 levels in EBC on days 1 and 2 were 24.05 $pg \cdot mL^{-1}$ (11.8–57.2) and 30.2 $pg \cdot mL^{-1}$ (14.4–53.2), respectively. The mean difference with coefficient of variability was 6.2±9.3 and all differences were within ±2 SD. The correlation between PGE_2 measurements on the 2 consecutive days was significant (r^2=0.619, p=0.006). The intraclass correlation coefficient for PGE_2 measurements was 0.79.

The stability of the PGE_2 in frozen samples showed significant differences between the four measurements (after 2 days: 23±11 $pg \cdot mL^{-1}$; after 1 week: 25±13 $pg \cdot mL^{-1}$; after 2 weeks: 24±14 $pg \cdot mL^{-1}$; after 3 weeks: 41±14 $pg \cdot mL^{-1}$; p=0.43 higher after 3 weeks).

The concentration of PGE_2 was significantly elevated in asthmatic smokers as compared to nonsmoking asthmatics and control subjects (40±21 *versus* 14.5±4.5 *versus* 11.7±3 $pg \cdot mL^{-1}$, respectively; p<0.0001; fig. 1). Further analysis showed that PGE_2 levels were significantly higher in asthmatic smokers compared to smoking and nonsmoking controls (40±21 *versus* 11.6±2 *versus* 11.7±4 $pg \cdot mL^{-1}$, respectively; p<0.0001; fig. 1). No significant difference was observed between asthmatic nonsmokers and both control smokers and nonsmokers (14.5±4.5 *versus* 11.6±2 *versus* 11.7±4 $pg \cdot mL^{-1}$, respectively; p=0.66; fig. 1). Concentration of PGE_2 was significantly elevated in patients with asthma as compared to the control group (27±20 *versus* 11.7±3 $pg \cdot mL^{-1}$; p<0.05). No significant difference was observed between smoking and nonsmoking controls (11.6±2 *versus* 11.7±4 $pg \cdot mL^{-1}$, respectively; p=0.9).

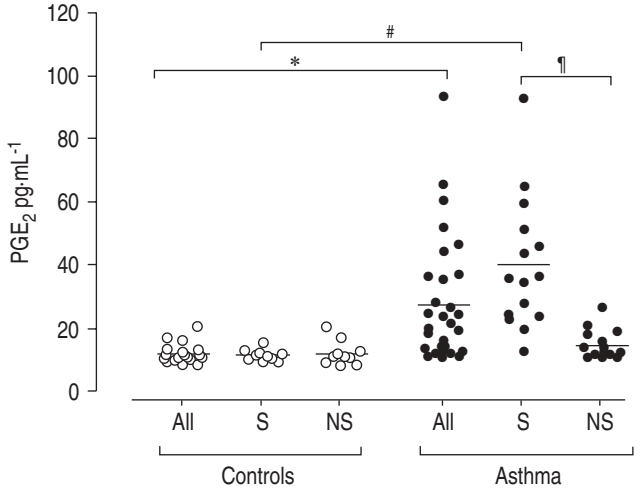

Fig. 1. – Prostaglandin (PG)E_2 concentration in exhaled breath condensate of control subjects (all n=20, smokers (S) n=10, nonsmokers (NS) n=10) and of patients with asthma (all n=30, smokers n=15, nonsmokers n=15). Individual and mean (—) values are shown. *: p<0.05; #: p=0.003; ¶: p=0.0006.

Correlations

No significant correlation was observed between PGE_2 values and PD20 to histamine in asthmatic patients (r_s=0.025, p=0.7). Accordingly, no significant correlation data were observed in the study subgroups (r_s=0.021, p=0.9 in asthmatic nonsmokers, r_s=0.11, p=0.12 in asthmatic smokers). No significant correlation was observed between smoking history (pack-yrs) and PGE_2 values (r_s=0.07, p=0.78).

Discussion

In this prospective, cross-sectional study, significant elevation of PGE_2 in the EBC of asthmatic smokers was found, as compared to nonsmoking asthmatics. Additionally, PGE_2 values did not predict the degree of bronchial hyperresponsiveness. The measurement seems to be repeatable but not stable after a period of 2 weeks.

Concerning the stability data presented in this study, it must be mentioned that no data are available in the literature regarding the storage of frozen PGE_2 samples in EBC. The current data suggest that there are significant alterations in the rate of PGE_2 concentrations over time, with a cut-off point at 2 weeks. This finding is hard to explain, but it might be partially supported by the *in vitro* formation of PGE_2 in the frozen samples that affect the stability of PGE_2 in the EBC samples over time. Such *in vitro* formation of PGE_2 has been described previously [19]. A plausible source of PGE_2 might be PGH_2, which is transformed to PGE_2 and PGD_2 through the action of prostaglandin isomerases [20].

As PGE_2 has a bronchodilatory and inhibitory effect on the inflammatory process at concentrations known to be present in the airways, impaired production of PGE_2 has been proposed to contribute to the pathogenesis of asthma. It would be expected that the measurement of PGE_2 in biological specimens might provide decreased levels compared to normal subjects. However, the present data and previous reports regarding nonsmoking asthmatics do not confirm the above hypothesis [9, 10, 21]. One explanation for the above findings is that only mild stable asthmatics were included in these studies. However, in one of the above studies [20], decreased concentrations of PGE_2 in sputum supernatant were found in acute severe asthma. One reasonable hypothesis is that, as the severity of the disease progresses and more severe patients are included, the values of PGE_2 might tend to decrease. Conversely, it could be argued that in more severe patients the levels of PGE_2 might have a tendency to increase in an attempt to control the increased inflammatory process.

One of the findings of the current study, which has not been investigated previously, is the effect of smoking. The results presented here show that smoking significantly increased the levels of PGE_2 in asthmatic subjects, even compared to normal smoking subjects. This might be explained by previous observations that cigarette smoke stimulates the formation of PGs in alveolar macrophages, that long-term

nicotine substitution diminishes the beneficial effects of smoking cessation due to the possible stimulatory effects of nicotine and cotinine on eicosanoid synthesis even *in vivo* [22], and, finally, that accumulation of cotinine, the major metabolite of nicotine, in foetal circulation contributes to production of PGE_2 [23].

The interesting finding was the significant difference in PGE_2 levels between asthmatic smokers and smoking controls, and the absence of a significant difference between smokers and nonsmoking controls. PGE_2 is largely produced by epithelial and airway smooth muscle cells, but all cells in the airways have the capacity to release prostanoids, and in particular macrophages [24]. Since cigarette smoke stimulates the formation of PGs from macrophages and considering all the above points together, the current authors believe that the significant difference in PGE_2 levels between asthmatic smokers and smoking controls may be attributed to the different activation state of macrophages in the two groups, indicating that the higher values of PGE_2 in asthmatics are mainly due to the activation of more macrophages and the greater release of PGE_2 from the macrophages. The non-significant difference between smoking and nonsmoking controls might be based on the inactivated state of airway cells, particularly macrophages, which possibly leads to minimal release of PGE_2. Although no specific correlation data are available in this study, the elevated levels of PGE_2 in asthmatic smokers might serve as an index of activation of inflammatory airway cells, particularly macrophages.

Regarding the relationship between PGE_2 concentration and its effect on bronchial hyperresponsiveness, as assessed by histamine challenge, no significant correlation was observed in asthmatic patients irrespective of their smoking habit. The absence of significant correlation might be based on the fact that PGE_2 inhibits challenges that work indirectly *via* neural pathways or mediator release but not those that act predominantly *via* a direct action on airway smooth muscle, such as histamine and metacholine [2, 3]. The above theory is partially supported by previous observations that PGE_2 inhibits acetylcholine release from cholinergic nerves [25], represents the ultimate mediator of airway smooth muscle relaxation produced by activation of sensory nerve inhibitory system [26], but fails to protect against bronchoconstriction induced by methacholine [27]. Another plausible explanation might be based on the theory that the inhibitory effect of PGE_2 in histamine-induced contraction appears to be concentration-dependent [28]. This means that the concentration of PGE_2 released from the airways in mild stable asthma is probably too low or inadequate in order to exert a significant effect on histamine-induced contractions.

PGE_2 has been shown to affect airway physiology by inhibiting both the early and late asthmatic response [3, 29], and the activation of mast cells, without resulting in significant bronchodilation. This means that it seems more plausible that the mechanisms of action of PGE_2 are anti-inflammatory rather than bronchodilatory. This is partially supported by previous observations that showed that PGE_2 inhibits many inflammatory events, including mast cell degranulation [9] and the expression of the cyclo-oxygenase pathway in the airways [30]. Conversely, its bronchodilatory effect seems to be limited [2, 3, 5]. All the above points together lead to the conclusion that exogenous administration or an increase in the endogenous production of PGE_2 might have a protective anti-inflammatory effect, but that it probably has a little, nonsignificant effect on the acute bronchoconstriction process.

In conclusion, the higher prostaglandin E_2 values in patients with mild asthma, as compared to normal controls, are mainly attributed to smoking habit, probably *via* increased production by activated inflammatory cells, particularly macrophages. The higher levels of endogenous prostaglandin E_2 do not have a protective role on the bronchospasm that is induced by histamine challenge testing.

References

1. Philipps RP, Stein SH, Roper LR. A new view of prostaglandin E_2 regulation of the immune response. *Immunol Today* 1991; 12: 349–352.
2. Pavord DI, Tattersfield AE. Bronchoprotective role for endogenous prostaglandin E_2. *Lancet* 1994; 344: 436–438.
3. Pavord DI, Wong CS, Williams J, Tattersfield AE. Effect of inhaled prostaglandin E_2 on allergen-induced asthma. *Am Rev Respir Dis* 1993; 148: 87–90.
4. Pasargiklian M, Bianco C, Allegra L, *et al.* Aspects of bronchial reactivity to prostaglandins and aspirin in asthmatic patients. *Respiration* 1977; 34: 79–91.
5. Kharitonov SA, Sapienza MA, Barnes PJ, Chung KF. Prostaglandins E_2 and F_{2a} reduce exhaled nitric oxide in normal and asthmatic subjects irrespective of airway caliber changes. *Am J Respir Crit Care Med* 1998; 158: 1374–1378.
6. Tetsuka T, Morrison AR. Tyrosine kinase activation is necessary for inducible nitric oxide synthase expression by interleukin-1β. *Am J Physiol* 1995; 269: 55–59.
7. Peters SP, Schulman ES, MacGlashan DW, Schleimer RP, Newball HH, Lichtenstein LM. Pharmacological and biochemical studies of human lung mast cells. *J Allergy Clin Immunol* 1982; 69: 150–158.
8. Kharitonov SA, Barnes PJ. Exhaled markers of pulmonary disease. *Am J Respir Crit Care Med* 2001; 163: 1693–1622.
9. Montuschi P, Barnes PJ. Exhaled leukotrienes and prostaglandins in asthma. *J Allergy Clin Immunol* 2002; 109: 615–620.
10. Antczak A, Montuschi P, Kharitonov S, Gorski P, Barnes PJ. Increased exhaled cysteinyl-leukotrienes and 8-isoprostane in aspirin-induced asthma. *Am J Respir Crit Care Med* 2002; 166: 301–306.
11. Hwang D, Chanmugam P, Boudreau M, Sohn KH, Stone K, Pryor WA. Activation and inactivation of cyclo-oxygenase in rat alveolar macrophages by aqueous cigarette tar extracts. *Free Radic Biol Med* 1999; 27: 673–682.
12. Global strategy for asthma management prevention. NIH Publication No. 02-3659. Bethesda, MD, National Institutes of Health, National Heart, Lung and Blood Institute, February 2002.
13. Sovijarvi AR, Malberg LP, Reinikminen K, Ryhila Poggins H. A rapid dosimetric method with controlled tidal breathing for histamine challenge. *Chest* 1993; 104: 164–170.
14. American Thoracic Society official statement. Guidelines for Methacholine and Exercise Challenge Testing - 1999. *Am J Respir Crit Care Med* 2000; 161: 309–329.
15. Ganas K, Loukides S, Papatheodorou G, Panagou P, Kalogeropoulos N. Total nitrite/nitrate in expired breath condensate of patients with asthma. *Respir Med* 2001; 95: 649–654.
16. Huszar E, Vass G, Vizi E, *et al.* Adenosine in exhaled breath condensate in healthy volunteers and in patients with asthma. *Eur Respir J* 2002; 20: 1393–1398.
17. Jobsis Q, Raatgeep HC, Schelleke SL, Hop WCJ, Hermans PW, de Jongste JC. Hydrogen peroxide in exhaled air of healthy children: reference values. *Eur Respir J* 1998; 12: 483–485.
18. Bland JM, Altman DG. Statistical method for assessing agreement between two methods of clinical measurement. *Lancet* 1986; 1: 307–310.
19. Finley RR, Bogert CL, Alberts DS, *et al.* Measurement of prostaglandin E_2 in rectal mucosa in human subjects: a method study. *Cancer Epidemiol Biomarkers Prev* 1995; 4: 239–244.
20. O′Byrne P. Prostanoids. *In*: Barnes PJ, Drazen JM, Rennard S,

Thompson NC, eds. Asthma and COPD. San Diego, Academic Press, 2002; pp. 221–226.
21. Pavord ID, Ward R, Woltmann G, Wardlaw AJ, Sheller JR, Dworski R. Induced sputum eicosanoid concentrations in asthma. *Am J Respir Crit Care Med* 1999; 160: 1905–1909.
22. Riutta A, Saareks V, Mucha I, Alanko J, Parviainen M, Vapaatalo H. Smoking cessation and nicotine substitution modulate eicosanoid synthesis *ex vivo* in man. *Naunyn Schmiedebergs Arch Pharmacol* 1995; 352: 102–107.
23. Rama Sastry BV, Hemontolor ME, Olenick M. Prostaglandin E_2 in human placenta: its vascular effects and activation of prostaglandin E_2 formation by nicotine and cotinine. *Pharmacology* 1999; 58: 70–86.
24. Barnes PJ, Chung KP, Page PC. Inflammatory mediators of asthma. An update. *Pharmacol Rev* 1998; 50: 515–596.
25. Zhao WW, Robinson NE, Yu MF. PGE_2 inhibits acetylcholine release from cholinergic nerves in canine airways. *Prostaglandins Leukot Essent Fatty Acids* 1994; 51: 347–355.
26. Szarek JL, Spurlock B, Gruetter CA, Lemke S. Substance P and capsaicin release prostaglandin E_2 from rat intrapulmonary bronchi. *Am J Physiol* 1998; 275: 1006–1012.
27. Pavord ID, Wisniewski A, Mathur R, Wahedna I, Knox A, Tattersfield AE. Effects of inhaled prostaglandin E_2 on bronchial reactivity to sodium metabisulphite and metacholine in subjects with asthma. *Thorax* 1991; 46: 633–637.
28. Knight DA, Stewart GA, Thompson PJ. Prostaglandin E_2 but not prostacyclin inhibits histamine-induced contraction of human bronchial smooth muscle. *Eur J Pharmacol* 1995; 272: 13–19.
29. Hartert TV, Dworski RT, Mellen BG, Oates JA, Murray JJ, Sheller JR. Prostaglandin $E_{(2)}$ decreases allergen-stimulated release of prostaglandin $D_{(2)}$ in airways of subjects with asthma. *Am J Respir Crit Care Med* 2000; 162: 637–640.
30. Bonazzi A, Bolla M, Buccellati C, *et al.* Effect of endogenous and exogenous prostaglandin E(2) on interleukin-1 beta-induced cyclooxygenase-2 expression in human airway smooth-muscle cells. *Am J Respir Crit Care Med* 2000; 162: 2272–2277.