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Anaphylaxis increases 8-*iso*-prostaglandin $F_{2\alpha}$ release from guinea-pig lung in vitro

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Abstract

8-*Iso*-prostaglandin $F_{2\alpha}$ release from isolated and perfused guinea-pig lung was measured by radioimmunoassay. 8-*Iso*-prostaglandin $F_{2\alpha}$ release was detectable under basal conditions and increased 10-fold during antigen-induced bronchoconstriction, concomitant with the increase of thromboxane B_2 and prostaglandin E_2. The anti-8-*iso*-prostaglandin $F_{2\alpha}$ serum used in the radioimmunoassay seems to be quite specific for this compound. Pretreatment of lungs with indomethacin (a non-selective inhibitor of cyclooxygenase) reduced 8-*iso*-prostaglandin $F_{2\alpha}$ release under basal conditions and completely abolished the increase observed during lung anaphylaxis. Pretreatment of lungs with NS 398 (*N*-[2-cyclohexyl]-4-nitrophenyl methanesulphonamide), a selective inhibitor of cyclooxygenase-2, did not change basal or antigen-induced 8-*iso*-prostaglandin $F_{2\alpha}$ release at all. We conclude that under basal conditions guinea-pig lung perfusates contain low levels of 8-*iso*-prostaglandin $F_{2\alpha}$-like immunoreactivity, which increase 10-fold during antigen-induced bronchoconstriction. This isoprostane seems to be derived from the cyclooxygenation of arachidonic acid via the constitutive form of cyclooxygenase.

Keywords: 8-*Iso*-prostaglandin $F_{2\alpha}$; Isoprostane; Lung, guinea-pig, isolated; Antigen-challenge; Radioimmunoassay

1. Introduction

F_2 isoprostanes are prostaglandin-like compounds, reportedly formed by free radical catalyzed peroxidation of arachidonic acid, independent of the action of cyclooxygenase (Morrow et al., 1990). They consist of isomers of the common prostaglandins and were reported for prostaglandin F_2, E_2 and D_2 series (Morrow et al., 1990, 1994). Isoprostanes are formed initially from arachidonic acid in situ in phospholipids and are putatively cleaved from the membrane by phospholipases A_2 (Morrow et al., 1992). 8-*Iso*-prostaglandin $F_{2\alpha}$ is the best characterized isoprostane and has been found to modulate platelet aggregation (Morrow et al., 1992) and to exert reduction in the glomerular filtration rate and renal blood flow (Takahashi et al., 1992). Moreover, a dose-dependent vasoconstrictor and bronchoconstrictor effect of this isoprostane was described in perfused lungs of rats (Kang et al., 1993), as well as in guinea pigs in vivo (Okazawa et al., 1997); these effects can be prevented by antagonists of the thromboxane receptor (Banerjee et al., 1992; Takahashi et al., 1992; Okazawa et al., 1997). 8-*Iso*-prostaglandin $F_{2\alpha}$ also displays smooth muscle constrictor activity in human and guinea-pig bronchi in vitro (Kawikova et al., 1996). It has been proposed that isoprostane measurement might offer a quantitative index of free radical generation; thus, 8-*iso*-prostaglandin $F_{2\alpha}$ has been considered a biomarker of oxidative stress in vivo in human diseases (see Patrono and FitzGerald, 1997 for a review). However, a cyclooxygenase-dependent isoprostane formation has been reported in human platelets (Praticò et al., 1995), human monocytes (Patrignani et al., 1996; Praticó and FitzGerald, 1996) and in diverse enzymatic and cellular systems as well as in vivo in humans (Klein et al., 1997).

Pulmonary allergic responses in guinea-pig lungs are associated with increased lipid peroxidation; previous studies have characterized the release of thromboxane and leukotrienes from anaphylactic guinea-pig lungs and found that thromboxane B_2 (the stable hydrolysis product of thromboxane A_2) is the most abundant cyclooxygenase product of arachidonic acid metabolism in anaphylactic lung perfusates (Robinson et al., 1984; Ciabattoni et al.,

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1993). The present study was conducted (1) to measure 8-*iso*-prostaglandin $F_{2\alpha}$-like-immunoreactivity release from isolated, ventilated and perfused guinea-pig lung under basal conditions and antigen-induced bronchoconstriction, and (2) to determine whether (and to what extent) this compound might be derived from the cyclooxygenation of arachidonic acid induced by cyclooxygenase-1 or -2 activation using selective and non-selective inhibitors of this enzyme. Thromboxane B_2 and prostaglandin E_2 release in lung perfusates was also measured as a biomarker of cyclooxygenase activation during anaphylactic response (Ciabattoni et al., 1993).

2. Materials and methods

2.1. Animal treatment

Male guinea-pigs of the Dunkin–Hartley strain (400–500 g) were sensitized with 1% ovalbumin solution, 20 mg being given intraperitoneally and 20 mg subcutaneously after 10 min. After 15–21 days, the animals were anesthetized with thiopental sodium (Pentothal, Abbott) 50 mg kg^{-1} injected i.p. Trachea was dissected in the upper cervical region, a tracheal cannula was inserted and a positive pressure ventilation was initiated. The chest was opened, 5000 I.U. heparin injected into the right ventricle of the heart and the inferior caval vein cut. The pulmonary artery was cannulated via an incision in the right ventricle and an outlet cannula was secured in the left atrium via an incision in the apex of the heart. The lung and the heart were removed and suspended in a Hugo Sachs Helektronik (March-Hugstetten, Germany) apparatus and perfused at a constant flow (5 ml min^{-1}) at 37°C with a Krebs–Ringer bicarbonate solution (gassed with 5% CO_2/95% O_2) injected through the pulmonary artery. Once suspended in the sealed chamber, the lungs were ventilated at 50 breaths min^{-1} by creating an alternating negative pressure (end-inspiration pressure: from -8 to -13 cm H_2O; end-expiration pressure: from -2 to -4 cm H_2O). The following parameters were registered: perfusion pressure, tidal volume, maximal inspiratory flow, maximal expiratory flow, end-inspiratory pressure, end-expiratory pressure, resistance and compliance.

Table 1
Respiratory parameters of guinea-pig lungs before and after antigen challenge with ovalbumin

	Basal	After ovalbumin challenge
Respiratory rate (bpm)	50	50
Maximal inspiratory flow (ml s^{-1})	20.54 ± 0.65	0.38 ± 0.02[a]
Maximal expiratory flow (ml s^{-1})	-15.02 ± 0.54	0.24 ± 0.02[a]
Tidal volume (ml)	4.66 ± 0.22	0.0 ± 0.0[a]
End inspiratory pressure (cm H_2O)	-10.94 ± 0.07	-10.98 ± 0.05
End expiratory pressure (cm H_2O)	-2.82 ± 0.04	-2.60 ± 0.03
Resistance (ml (cm $H_2O)^{-1}$ s^{-1})	0.52 ± 0.02	1000 (out of scale)
Compliance (ml (cm $H_2O)^{-1}$)	0.46 ± 0.04	-1000 (out of scale)
Perfusion pressure (cm H_2O)	12.06 ± 0.48	21.48 ± 0.75[a]

Numbers are mean ± S.E.M.; $n = 8$; [a] $P < 0.01$.

Table 2
Thromboxane (TX) B_2, prostaglandin (PG) E_2 and 8-*iso*-prostaglandin $F_{2\alpha}$ release from isolated, ventilated and perfused guinea-pig lung before and after antigen challenge with ovalbumin

	TXB_2 (ng min^{-1})	PGE_2 (ng min^{-1})	8-*iso*-$PGF_{2\alpha}$ (pg min^{-1})
Basal	2.61 ± 0.34	0.19 ± 0.04	42.5 ± 8.3
Basal	2.43 ± 0.20	0.20 ± 0.04	42.8 ± 4.7
Basal	2.41 ± 0.21	0.21 ± 0.04	43.6 ± 5.3
Ovalbumin challenge			
First collection	11.75 ± 0.81[a]	1.01 ± 0.02[a]	427.2 ± 64.7[a]
Wash-out	6.97 ± 0.78[a]	0.64 ± 0.04[a]	262.0 ± 67.5[a]
Wash-out	5.88 ± 0.76[a]	0.47 ± 0.04[a]	161.5 ± 21.2[a]
Wash-out	3.92 ± 0.89	0.24 ± 0.03	99.2 ± 13.0
Wash-out	2.98 ± 0.53	0.19 ± 0.02	47.0 ± 5.2

Samples of lung perfusates were collected every 2 min. Values are expressed as means ± S.E.M., and those marked with an [a] differ significantly from the mean basal value. [a] $P < 0.01$, $n = 8$.

2.2. Sample collection and ovalbumin challenge

The lung perfusion solution was removed from the bottom of the chamber by a peristaltic pump connected to a fraction collector. Samples of the perfusate were collected every 2 min. The solution was chilled on ice, added to 4-hydroxy-2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) to yield a final dilution of 10^{-3} M, centrifuged at 4°C and stored at −25°C until assayed. The lungs were allowed to equilibrate for at least 10 min and challenged with 0.1% ovalbumin solution given through the pulmonary artery (5 mg in 1 min). Some of the sensitized lungs were pretreated with indomethacin (10^{-6} M) or the selective cyclooxygenase-2 inhibitor NS 398 (*N*-[2-cyclohexyl]-4-nitrophenyl methanesulphonamide) (10^{-6} M) for at least 20 min before exposure to ovalbumin.

2.3. Analytical methods

8-*Iso*-prostaglandin $F_{2\alpha}$ was extracted by running 5 ml of lung perfusate on SEP-PAK C-18 cartridges (Waters, Associated, Milford, MA) and eluting with ethyl acetate. The dried samples were recovered in 0.5 ml of assay buffer. Recovery of tritium labeled 8-*iso*-prostaglandin $F_{2\alpha}$ averaged 98.3 ± 0.42% (mean ± S.E.M., $n = 12$). The elu-

ates were assayed for 8-*iso*-prostaglandin $F_{2\alpha}$ by radioimmunoassay, as previously described (Antiserum Rab. 1, Wang et al., 1995), at a final dilution 1:6 into the assay mixture. Under these conditions the detection limit in lung perfusate solution was 1.2 pg ml^{-1}. Perfusate levels of thromboxane B_2 and prostaglandin E_2 were measured using a specific radioimmunoassay (Ciabattoni et al., 1993). Validation of radioimmunoassay measurements was sought by comparison between two different anti 8-*iso*-prostaglandin $F_{2\alpha}$ sera (Antiserum Rab. 1 and Antiserum L9 described by Wang et al., 1995) and reverse-phase high-performance liquid chromatography (RP-HPLC) analysis of 8-*iso*-prostaglandin $F_{2\alpha}$-like immunoreactivity. For this purpose, a pool of samples was extracted and subjected to RP-HPLC with the solvent system acetonitrile:water:acetic acid (23:77:0.1) at a flow rate of 1 ml min^{-1} to separate 8-*iso*-prostaglandin $F_{2\alpha}$ from other prostanoids. The eluates collected every min were dried, reconstituted with buffer and subjected to radioimmunoassay at different dilutions with the two different antisera. The retention times of 8-*iso*-prostaglandin $F_{2\alpha}$ and other eicosanoids were determined either by injecting separately 0.1–0.2 μg of the standard compounds and subsequent ultraviolet analysis of eluates, or by injecting about 50,000 c.p.m. of the tritium labeled compounds and counting consecutively 1 ml aliquots of the column eluate.

2.4. Materials

The following compounds were used: thiopental sodium (Pentothal, Abbott), 4-hydroxy-TEMPO (Sigma), ovalbumin (Merck), indomethacin (Merck Sharp and Dohme), NS 398 (Cayman Chemicals). SEP-PAK C-18 cartridges were purchased from Waters Associated, Milford, MA.

Table 3
Specificity of anti-8-*iso*-$PGF_{2\alpha}$ sera used for radioimmunoassay

Ligand	Cross-reactivity of antisera (%)	
	L9	Rab. 1
8-*Iso*-$PGF_{2\alpha}$	100	100
8-*Iso*-PGE_2	0.08	7.7
$PGF_{2\alpha}$	0.02	0.24
TXB_2	0.01	< 0.02
6-Keto-$PGF_{1\alpha}$	< 0.01	< 0.02
PGE_2	< 0.01	0.56
2,3-Dinor-6-keto-$PGF_{1\alpha}$	< 0.01	< 0.01
2,3-Dinor-TXB_2	0.03	< 0.01
11-Dehydro-TXB_2	N.T.	< 0.01
PGD_2	0.2	0.1
6,15-Diketo-$PGF_{1\alpha}$	N.T.	< 0.001
13,14-Dihydro-15-keto-$PGF_{2\alpha}$	N.T.	< 0.001
13,14-Dihydro-15-keto-PGE_2	N.T.	< 0.001

Cross-reactivities were determined after addition of either homologous (8-*iso*-$PGF_{2\alpha}$) or heterologous (other eicosanoids) ligands to the antibody–tracer complex. Displacement of 50% initial binding was determined for the different compounds and relative % was expressed as the concentration of homologous/concentration of heterologous ligand × 100.
N.T. = not tested.
Abbreviations as in Table 2.

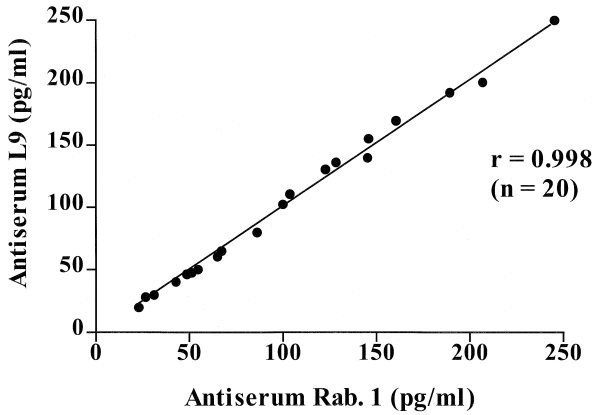

Fig. 1. Validation of immunological measurement of 8-*iso*-prostaglandin (PG) $F_{2\alpha}$. Correlation between measurements of lung perfusates by radioimmunoassay using two different antisera directed against 8-*iso*-prostaglandin $F_{2\alpha}$ with a slight difference in cross-reactivities (antiserum Rab. 1 and antiserum L9 described by Wang et al., 1995).

2.5. Statistical analysis

Group means for all data were subjected to parametric analysis of variance for multiple comparisons and Student's *t*-test for single comparisons. Results are reported as means ± S.E.M. $P < 0.05$ was considered statistically significant.

3. Results

In most of the sensitized lungs (about 80%), the ovalbumin challenge induced bronchoconstriction lasting the entire period of observation (30 min). Only those guinea-pig

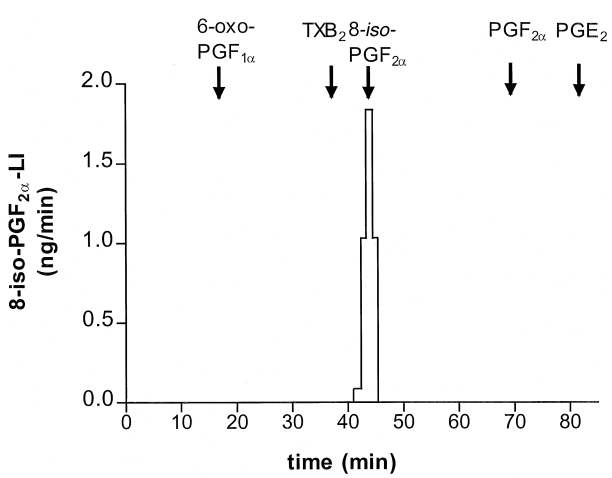

Fig. 2. RP-HPLC analysis of 8-*iso*-prostaglandin $F_{2\alpha}$-like immunoreactivity (LI) in pool of samples of lung perfusates. Eluates were collected every minute. Arrows indicate the retention times of 6-oxo-prostaglandin $F_{1\alpha}$, thromboxane (TX) B_2, 8-*iso*-prostaglandin $F_{2\alpha}$, prostaglandin $F_{2\alpha}$ and prostaglandin E_2.

lungs that underwent bronchoconstriction were considered. Non-sensitized lungs were used as controls. The anaphylactic reactions were manifested by a dramatic reduction (to unmeasurable levels) within 1 or 2 min of the tidal volume, the respiratory flow and the compliance (calculated on the basis of the previous parameters), while airway resistance increased over the detection limit of the instrument (Table 1). The response was accompanied by an increase in perfusate levels of 8-*iso*-prostaglandin $F_{2\alpha}$, prostaglandin E_2 and thromboxane B_2 (Table 2), which returned to baseline within 8 min after antigen-challenge. No increase in the release of these isoprostanes by control lungs was observed.

Validation of the analysis of 8-*iso*-prostaglandin $F_{2\alpha}$ was sought by three different criteria. (1) The extracts were quantified by radioimmunoassay using two antisera with different cross-reactivities (Table 3). For this purpose 20 extracts of lung perfusates were simultaneously tested with the two antisera. Remarkably similar concentrations were measured using either antiserum ($r = 0.998$, $n = 20$, $P < 0.001$; Fig. 1). (2) RP-HPLC separation of a pool of samples and assay with antiserum Rab. 1 showed a single peak of immunoreactivity that co-eluted with 8-*iso*-prostaglandin $F_{2\alpha}$ standard, thus indicating that the unknown 8-*iso*-prostaglandin $F_{2\alpha}$-like immunoreactive material(s) recognized by antiserum has identical chromatographic behavior with authentic 8-*iso*-prostaglandin $F_{2\alpha}$ (Fig. 2). (3) The peak of immunoreactivity that co-eluted with 8-*iso*-prostaglandin $F_{2\alpha}$ standard after RP-HPLC was assayed over a 6- to 150-fold range of dilution with both antisera. As shown in Fig. 3, the percent bound values corresponding to the increasing volumes of the assayed material fall along the standard curve for both radioimmunoassays. This indicates that in these assays the immunochemical behavior of the endogenous substance can not be distinguished from that of authentic 8-*iso*-prostaglandin $F_{2\alpha}$.

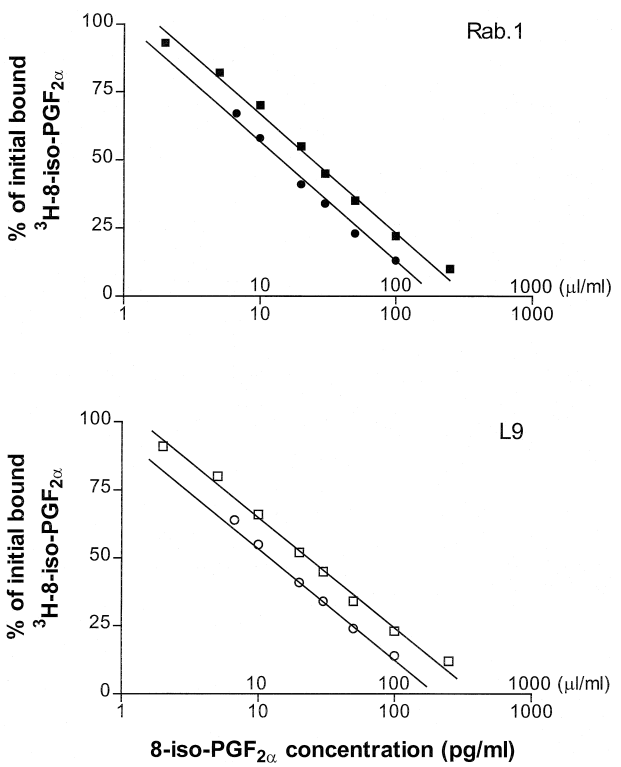

Fig. 3. Verification of 8-*iso*-prostaglandin $F_{2\alpha}$ radioimmunoassay. Standard curves in buffer obtained with antiserum Rab. 1 (upper panel, closed squares) and antiserum L9 (lower panel, open squares). The percentage change of initial binding of ^{3}H-8-*iso*-prostaglandin $F_{2\alpha}$ is plotted on a logit scale as a function of concentration of unlabeled 8-*iso*-prostaglandin $F_{2\alpha}$. The peak of immunoreactivity that co-eluted with 8-*iso*-prostaglandin $F_{2\alpha}$ standard after RP-HPLC was assayed over a 6- to 150-fold range of dilution with antiserum Rab. 1 (upper panel, closed circles) and L9 (lower panel, open circles). The percentage change of initial binding of ^{3}H-8-*iso*-prostaglandin $F_{2\alpha}$ is plotted as a function of the increasing volumes (on the internal abscissa scale) of the assayed material.

Table 4
Effects of indomethacin treatment (10^{-6} M) on eicosanoid release from isolated perfused and ventilated lung of guinea pig under basal conditions (control) and immediately after antigen challenge

	8-*iso*-$PGF_{2\alpha}$ (pg min^{-1})	TXB_2 (ng min^{-1})	PGE_2 (ng min^{-1})
Basal conditions	8.6 ± 2.3	0.182 ± 0.02	0.021 ± 0.01
After ovalbumin challenge	9.3 ± 3.5	0.186 ± 0.03	0.020 ± 0.01

Values are expressed as means ± S.E.M., $n = 5$.
Abbreviations as in Table 2.

In five sensitized lungs undergoing lung anaphylaxis, indomethacin treatment almost abolished 8-*iso*-prostaglandin $F_{2\alpha}$ release under control conditions and prevented the increase observed after antigen challenge (Table 4). An identical pattern was observed with thromboxane B_2 and prostaglandin E_2 release (Table 4). Indomethacin did not modify the maximal reduction of respiratory parameters, although it slightly increased the time required to achieve maximal bronchoconstriction, as previously described (Ciabattoni et al., 1993).

Table 5
Effects of NS 398 treatment (10^{-6} M) on eicosanoid release from isolated perfused and ventilated lung of guinea pig under basal conditions (control) and immediately after antigen challenge

	8-*iso*-$PGF_{2\alpha}$ (pg min^{-1})	TXB_2 (ng min^{-1})	PGE_2 (ng min^{-1})
Basal conditions	46.0 ± 6.4	2.64 ± 0.33	0.202 ± 0.03
After ovalbumin challenge	413.8 ± 59.0[a]	11.52 ± 0.44[a]	1.09 ± 0.02[a]

Values are expressed as means ± S.E.M. [a] $P < 0.01$, when compared to basal values; $n = 5$.
Abbreviations as in Table 2.

Five lungs challenged with ovalbumin after NS 398 treatment underwent bronchoconstriction. In these lungs the selective cyclooxygenase-2 inhibitor did not modify the basal release of 8-*iso*-prostaglandin $F_{2\alpha}$ or the increase observed during the bronchoconstrictor response (Table 5). A similar pattern was observed with thromboxane B_2 and prostaglandin E_2 release (Table 5). No change in the reduction of respiratory parameters after NS 398 treatment was observed.

4. Discussion

Our findings show that, under basal conditions, guinea-pig lung perfusates contain detectable levels of 8-*iso*-prostaglandin $F_{2\alpha}$, which increase about 10-fold during antigen-induced bronchoconstriction. In view of the complex and perhaps incompletely identified spectrum of structurally related compounds derived from arachidonic acid metabolism, qualitative validation of radioimmunoassay measurement of 8-*iso*-prostaglandin $F_{2\alpha}$ was sought through studies of immunological and chromatographic behavior of the measured material. Demonstration of identical immunological behavior of the unknown substance with the standard used for its measurement is a necessary though not sufficient criterion of specificity, although the use of different antisera reinforces the validity of measurement, since every antiserum possesses a unique immunological profile of cross-reactivity. Demonstration of identical chromatographic behavior of the unknown immunoreactivity with a reference standard can be of greater importance, although limited by the possible existence of different compounds behaving in an indistinguishable fashion in certain solvent systems. We characterized 8-*iso*-prostaglandin $F_{2\alpha}$-like immunoreactivity by RP-HPLC and found a single peak co-eluting with authentic 8-*iso*-prostaglandin $F_{2\alpha}$. Although this is not a final criterion of specificity, our data strongly suggest that the immunoreactive material detected in lung perfusates is represented by authentic 8-*iso*-prostaglandin $F_{2\alpha}$. On the other hand, only the comparison with an independent assay method, such as gas chromatography/mass spectrometry, could provide definitive evidence for the identification of 8-*iso*-prostaglandin $F_{2\alpha}$-like immunoreactivity.

In agreement with our previous studies, the release of thromboxane B_2, prostaglandin E_2 and other eicosanoids (not reported in the present paper) also increases during lung anaphylaxis, thromboxane B_2 being the most abundant product of arachidonate cyclooxygenation (Ciabattoni et al., 1993).

Since 8-*iso*-prostaglandin $F_{2\alpha}$ release is known to increase under conditions of oxidative stress, we performed experiments to determine whether (and to what extent) 8-*iso*-prostaglandin $F_{2\alpha}$ might be derived from the cyclooxygenation of arachidonic acid induced by cyclooxygenase-1 or -2 activation by using a non-selective (indomethacin) cyclooxygenase inhibitor and a selective cyclooxygenase-2 inhibitor (NS 398). Indomethacin pretreatment (10^{-6} M) of perfused lungs, although not preventing the bronchoconstrictor response, strongly reduced 8-*iso*-prostaglandin $F_{2\alpha}$ as well as thromboxane B_2 and prostaglandin E_2 release under basal conditions and abolished the increase observed after ovalbumin challenge. Differently, the pretreatment of lungs with the selective cyclooxygenase-2 inhibitor at a dose (10^{-6} M) nearly completely inhibiting this enzyme in vitro (Panara et al., 1995), did not modify basal eicosanoid release or the increase observed after ovalbumin challenge. These data strongly suggest that 8-*iso*-prostaglandin $F_{2\alpha}$ measured in lung perfusates is derived from the cyclooxygenation of arachidonic acid induced by cyclooxygenase-1 activation, though it accounts for a minor component of cyclooxygenase activity as compared to thromboxane B_2 and prostaglandin E_2. Moreover, our data suggest that also prostaglandin E_2 and thromboxane B_2 synthesis is derived from the constitutive isoform of cyclooxygenase activity in lung anaphylaxis.

In contrast to classic prostaglandins, which are formed through the action of prostaglandin-synthase isozymes from free arachidonic acid, F_2 isoprostanes were originally reported to be formed in situ from the fatty acid backbone esterified in membrane phospholipids and to be released in response to cellular activation, presumably through a phospholipase-mediated mechanism. However, in addition to a cyclooxygenase-independent mechanism of formation, there is recent evidence that 8-*iso*-prostaglandin $F_{2\alpha}$, unlike other F_2 isoprostanes, can be produced as a minor product of the cyclooxygenase activity of cyclooxygenase-1 in human platelets and other cellular systems (Praticò et al., 1995; Klein et al., 1997). Moreover, induction of cyclooxygenase-2 in human monocytes was associated with cyclooxygenase-dependent formation of 8-*iso*-prostaglandin $F_{2\alpha}$ (Patrignani et al., 1996; Praticó and FitzGerald, 1996). Although the contribution of cyclooxygenase-dependent mechanisms to the formation of 8-*iso*-prostaglandin $F_{2\alpha}$ in vivo appears to be negligible under physiological circumstances, the role played by different mechanisms of formation should be explored in different pathophysiologic settings.

8-*Iso*-prostaglandin $F_{2\alpha}$ is a potent vasoconstrictor (Takahashi et al., 1992) and induces DNA synthesis in vascular smooth muscle cells, perhaps through interaction with receptors that are distinct from but closely related to prostaglandin H_2/thromboxane A_2 receptors (Fukunaga et al., 1993). In addition, pulmonary artery injection of 8-*iso*-prostaglandin $F_{2\alpha}$ induces a dose-dependent bronchoconstriction in the in situ isolated, perfused lungs of rats (Kang et al., 1993), but not in those of rabbits (Banerjee et al., 1992; Hill et al., 1997). 8-*Iso*-prostaglandin $F_{2\alpha}$ also displays smooth muscle constrictor activity in human and guinea-pig bronchi in vitro (Kawikova et al., 1996) and induces airflow obstruction and airway

plasma exudation in vivo in guinea pigs (Okazawa et al., 1997). In light of these effects, which are caused by 8-*iso*-prostaglandin $F_{2\alpha}$ interaction with thromboxane receptors (Kawikova et al., 1996; Okazawa et al., 1997), it is possible that this isoprostane plays a role in the pathogenesis of lung anaphylaxis in some species beside other bronchoconstrictor mediators, such as thromboxane and leukotrienes. However, our data do not support a primary role for 8-*iso*-prostaglandin $F_{2\alpha}$ in the antigen challenge-induced bronchoconstriction. In fact, indomethacin pretreatment abolishes the isoprostane peak observed during anaphylaxis but not the concomitant bronchoconstriction. Furthermore, considering that 8-*iso*-prostaglandin $F_{2\alpha}$ formation accounts only for 3–4% of cyclooxygenase activity during anaphylaxis, the role of this isoprostane in the pathophysiology of the antigen-induced bronchoconstriction seems to be limited.

Further studies are necessary (1) to identify the cellular source of 8-*iso*-prostaglandin $F_{2\alpha}$ in the lung, (2) to determine whether reactive oxygen species (such as free radicals) might induce 8-*iso*-prostaglandin $F_{2\alpha}$ generation, (3) to establish the pathophysiological relevance of this compound in lung anaphylaxis, and (4) to investigate the possible role of this isoprostane in human pathological conditions characterized by altered airway reactivity, such as asthma.

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