

Effects of selective and non-selective COX inhibitors on antigen-induced release of prostanoid mediators and bronchoconstriction in the isolated perfused and ventilated guinea pig lung

Ewa Selg, Lena Låstbom, Åke Ryrfeldt, Maria Kumlin, Sven-Erik Dahlén*

*Experimental Asthma and Allergy Research, The National Institute of Environmental Medicine, Division of Physiology,
Centre for Allergy Research, Karolinska Institutet, Box 287, SE-171 77 Stockholm, Sweden*

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Abstract

The contribution of cyclooxygenase (COX)-1 and COX-2 in antigen-induced release of mediators and ensuing bronchoconstriction was investigated in the isolated perfused guinea pig lung (IPL). Antigen challenge with ovalbumin (OVA) of lungs from actively sensitised animals induced release of thromboxane (TX)A₂, prostaglandin (PG)D₂, PGF_{2α}, PGI₂ and PGE₂, measured in the lung effluent as immunoreactive TXB₂, PGD₂-MOX, PGF_{2α}, 6-keto PGF_{1α} and PGE₂, respectively. This release was abolished by the non-selective COX inhibitor flurbiprofen (10 μM). In contrast, neither the selective COX-1 inhibitor FR122047 nor the selective COX-2 inhibitor celecoxib (10 μM each) significantly inhibited the OVA-induced bronchoconstriction or release of COX products, except for PGD₂. Another non-selective COX inhibitor, diclofenac (10 μM) also significantly inhibited antigen-induced bronchoconstriction. The data suggest that both COX isoenzymes, COX-1 and COX-2 contribute to the immediate antigen-induced generation of prostanoids in IPL and that the COX-1 and COX-2 activities are not associated with different profiles of prostanoid end products.

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1. Introduction

The early asthmatic reaction (EAR), manifested as airway narrowing, occurs within minutes after allergen inhalation by asthmatic subjects and resolves within 2–3 h. The EAR is caused by the release of bronchoconstrictor mediators in particular from mast cells activated by allergen [1].

The cysteinyl-leukotrienes (CysLTs), LTC₄, LTD₄ and LTE₄, are the predominant mediators responsible for the EAR. Treatment with drugs that inhibit the cysteinyl-leukotriene type 1 receptor (CysLT₁)-mediated effects [2–5] or the biosynthesis of CysLTs [6–9] attenuates the EAR. Inhibition of the histamine H₁

receptor-mediated effects has been shown to attenuate the EAR although to a lesser extent than antileukotriene drugs [5,10,11], indicating a role also of histamine as mediator of the EAR. However, combined antileukotriene and antihistamine treatment did not completely abolish the EAR [5], implicating that other mediators also contribute. Three main candidates are cyclooxygenase (COX)-derived prostanoids, thromboxane (TX)A₂ and prostaglandins D₂ and F_{2α} (PGD₂ and PGF_{2α}) that are released during the EAR [12–14] and that all exert bronchoconstrictive effects in human airways by activation of the thromboxane receptor (TP) [15].

Our previous pharmacological investigation of mediators of the immediate antigen-induced bronchoconstriction in the guinea pig isolated perfused and ventilated lung (IPL) documented a pivotal role of

*Corresponding author. Tel.: +46 8 52 48 72 03; fax: +46 8 300 619.
E-mail address: Sven-Erik.Dahlen@ki.se (S.-E. Dahlén).

presumably mast cell-derived CysLTs and histamine [16]. The IPL of this particular species is therefore considered a relevant experimental model for studies of the EAR. We also showed that the COX inhibitor diclofenac by itself inhibited the antigen-induced bronchoconstriction [16], implicating that COX products mediated one component of the antigen response in IPL. In addition, our previous findings specifically implicated involvement of bronchoconstrictive COX products acting on the TP receptor, as the airway response to antigen in IPL was abolished when a TP receptor antagonist was added to the perfusate together with an antileukotriene and an antihistamine [16].

The aim of the present study was to evaluate the specific role of the two COX isoenzymes, COX-1 and COX-2, in the antigen-induced generation of prostanooids and the ensuing bronchoconstriction in the IPL. The role of COX-1 and COX-2 in the immediate airway response to antigen has not been studied previously in this model, nor to any great extent in other models with relevance to asthma. This information is important with respect to the effects of NSAIDs and coxibs (selective COX-2 inhibitors) in asthmatic airways. We therefore investigated the effects of selective inhibition of COX-1 and COX-2 on the antigen-induced bronchoconstriction and release of prostanoids.

2. Materials and methods

2.1. Animal sensitisation

The experiments were approved by the local Ethical Review Board (N127/04). Male Dunkin Hartley guinea pigs weighing 200–250 g were sensitised at least 4 weeks prior to experiments to chicken egg albumin grade II (ovalbumin, OVA) (Sigma Chemicals Co., St. Louis, MO, USA) by one i.p. and one s.c. injection of 10 mg of OVA dissolved in 0.4 ml 0.9% NaCl and Al(OH)₃ hydrogel 1:1, v:v (Superfos Biosector, Frederikssund, Denmark).

2.2. Isolated perfused and ventilated guinea pig lung

The guinea pigs weighing 500–900 g were used. Whole lungs were prepared as described previously [16]. The lungs were challenged with antigen (OVA) administered as bolus injections in a constant volume (0.1 ml) of 0.9% NaCl into the perfusate inlet catheter placed in the pulmonary artery. The lungs were pretreated 20 min with 10 μ M flurbiprofen, diclofenac, celecoxib or FR122047, added to perfusate reservoir. Stock solutions of drugs were made in 95% ethanol (flurbiprofen and diclofenac), dimethylsulphoxide (FR122047), or polyethylene glycol MW 400 and 0.9% NaCl 2.33:1, v:v (celecoxib). All used drugs were added from the stock to

the perfusion medium in 1000-fold dilution or more, to avoid solvent effects on preparation.

Tracheal airflow was measured with a heated pneumotachograph (Hans Rudolph, Kansas City, MO, USA) connected to the transducer in the EMKA system (EMKA Technologies, Paris, France) that measured also chamber pressure. Lung-function parameters: airway conductance and dynamic compliance were calculated and recorded by a computerised data acquisition system with software IOX (EMKA Technologies). The baseline values for airway conductance, dynamic compliance and perfusion flow were $57 \pm 12 \text{ ml s}^{-1} \text{ kPa}^{-1}$, $6 \pm 2 \text{ ml kPa}^{-1}$ and $25 \pm 3 \text{ ml min}^{-1}$ (mean $\pm$ SD, $n = 65$), respectively. The peak effects on airway conductance and dynamic compliance were evaluated as % of baseline values. Since changes in dynamic compliance followed similar pattern as changes in airway conductance throughout all experiments, only the values of airway conductance are presented. Bronchoconstriction in the preparation is expressed as % decreases in airway conductance related to baseline.

2.3. Measurements of antigen-induced mediator release

The lung effluent was collected on ice in 30 s intervals, during 1 min immediately before and 5 min after challenge of the lungs with a single dose of antigen (OVA). The effluent samples were stored at -70°C prior to analyses of TXB₂ (a stable metabolite of TXA₂), PGD₂, PGF_{2 α} , PGE₂, 6-keto PGF_{1 α} (a stable metabolite of PGI₂) and histamine.

The contents of TXB₂, PGD₂, PGF_{2 α} , PGE₂ and 6-keto PGF_{1 α} were measured by enzyme immunoassay. The respective acetyl cholinesterase (AChE)-linked tracers were used in the assays. The detection limits were: 13 pg ml⁻¹ for TXB₂, 3.6 pg ml⁻¹ for PGD₂-MOX, 9 pg ml⁻¹ for PGF_{2 α} , 15 pg ml⁻¹ for PGE₂ and 11 pg ml⁻¹ for 6-keto PGF_{1 α} .

For analysis of TXB₂ a rabbit polyclonal antiserum (anti-TXB₂ rabbit IgG) with cross-reactivity to 2,3-dinor TXB₂ (9.9%), PGD₂ (0.53%), 11-dehydro TXB₂ (0.42%), PGF_{2 α} (0.25%) and PGE₂ (0.09%) was used.

Prior to analysis of PGD₂, the effluent samples were derivatised with methoxylamine-HCl in order to convert PGD₂ to a stable methoxime derivative (PGD₂-MOX). The samples were analysed for PGD₂-MOX using a rabbit polyclonal antiserum (anti-PGD₂-MOX rabbit IgG) with cross-reactivity to unmethoximated PGD₂ (0.2%), and <0.01% to PGE₂-MOX, TXB₂-MOX and PGF_{2 α} .

For analysis of PGF_{2 α} a rabbit polyclonal antiserum (anti-PGF_{2 α} rabbit IgG) was used, with cross-reactivity to PGF_{1 α} (61%), PGF_{3 α} (21%), 20-hydroxy PGF_{2 α} (5.3%), PGE₂ (2.6%) and PGD₂ (1.2%).

For analysis of PGE₂ a mouse monoclonal antiserum (anti-PGE₂ mouse IgG) was used, with cross-reactivity

to 8-iso PGE₂ (37.4%), 8-iso PGF_{2α} (0.025%), 13,14-dihydro-15-keto PGE₂ (0.02%), and <0.01% to PGD₂ and TXB₂.

For analysis of 6-keto PGF_{1α} a rabbit polyclonal antiserum (anti-6-keto PGF_{1α} rabbit IgG) was used, with cross-reactivity to PGF_{1α} (11%), 2,3-dinor-6-keto PGF_{1α} (5%), 13,14-dihydro-15-keto PGF_{1α} (3%), PGE₂ (2%), 6,15-diketo-13,14-dihydro PGF_{1α} (2%), PGF_{2α} (0.4%), TXB₂ (0.05%) and PGD₂ (<0.01%).

For analysis of histamine, a spectrofluorimetric assay was used. Briefly, the samples were alkalisied with 1 M NaOH and treated with 1% *o*-phthalaldehyde in methanol in order to produce fluorophores with histamine. The reaction was stopped after 4 min by acidification of the samples with 3 M HCl. Fluorescence readings were made in spectrophotofluorimeter at the excitation wavelength 360 nm and the emission wavelength 450 nm. Relative fluorescence intensities were transformed to histamine concentrations by the use of a standard calibration curve for histamine (0.9–1000 ng ml⁻¹). The detection limit was 1.9 ng ml⁻¹.

Prior to analysis of LTC₄, the solid phase eicosanoid extraction was performed directly after collection of the effluent samples, using C18 Isolute columns (100 mg/6 ml) (International Sorbent Technology, Mid Glamorgan, UK). The lung effluent samples were spiked with 10 000 d.p.m. [³H]-LTE₄, applied as internal standard. The samples were adjusted to pH 3–4 and applied to Isolute columns preconditioned with methanol and water. After passage of the samples, the columns were washed with water pH 3 and the analyte was eluted with methanol. The methanol fractions were stored at -70 °C, before the methanol was evaporated under nitrogen and the residue was resuspended in 500 μl of enzyme immunoassay buffer. An aliquot was counted by liquid scintillation for estimation of recovery. The mean overall recovery for all experiments was 53 ± 10% (mean ± SD, *n* = 22).

The content of LTC₄ was measured by enzyme immunoassay, using a rabbit polyclonal antiserum (anti-CysLT rabbit IgG) with cross-reactivity to LTC₄ (100%), LTD₄ (100%), LTE₄ (67%), LTD₅ (61%), LTC₅ (54%), LTE₅ (41%), *N*-acetyl LTE₄ (10.5%) and <0.01% to PGD₂, PGE₂, PGF_{2α}, LTA₄, LTB₄ and 6-keto PGF_{1α}. As AChE-linked LTC₄ was used as tracer and a standard calibration curve for LTC₄ was used, all data are expressed as LTC₄ equivalents. The detection limit was 13 pg ml⁻¹. The amounts of LTC₄ detected by enzyme immunoassay were corrected for the mean recovery calculated for each experiment. The levels of mediators in the lung effluent samples collected before the antigen challenge were close to or below the detection limit of the analytical methods. Control challenge with OVA up to 10 000 μg of the lungs from non-sensitised animals did not elicit bronchoconstriction.

2.4. Chemicals and drugs

All chemicals used in the study were of analytical grade and purchased from Sigma (St. Louis, MO, USA) and other commercial suppliers. COX inhibitors were purchased from Cayman Chemicals (Ann Arbor, MI, USA) and SynphaBase AG (Basel, Switzerland). All reagents for enzyme immunoassay analyses of TXB₂, PGD₂, PGF_{2α}, PGE₂, 6-keto PGF_{1α} and LTC₄ were purchased from Cayman Chemicals.

2.5. Statistical analysis

Data in figures are given as mean ± SD.

The obtained experimental data were statistically evaluated by Student's *t*-test for paired and unpaired observations. The observed differences were considered significant when *p* < 0.05 was obtained.

3. Results

3.1. Antigen-induced release of histamine, CysLTs and COX products

In initial experiments, the effects of three different doses of OVA (100, 1000 or 10 000 μg) on the release of histamine, CysLTs and COX products were tested in order to establish dose-response (Fig. 1). However, the amounts of histamine, LTC₄, TXA₂ (measured as immunoreactive TXB₂) and PGD₂, released in response to the challenge with 100 and 1000 μg of OVA, were not significantly different, whereas 10 000 μg of OVA produced a substantially greater release of all mediators (Fig. 2). The relative ratios between the total amount of LTC₄ and that of histamine, TXA₂ and PGD₂, released in response to the challenges with the three different doses of OVA are presented in Fig. 2. Similar to a previous study [17], there was a trend that relatively more histamine and TXA₂ was released by the highest dose of OVA, as compared to LTC₄ and PGD₂.

3.2. Effects of COX inhibitors on antigen-induced release of COX products

The release of COX products in response to antigen was further characterised using the most effective dose of OVA (10 000 μg). Release of TXA₂ (measured as immunoreactive TXB₂), PGD₂, PGF_{2α}, PGI₂ (measured as immunoreactive 6-keto PGF_{1α}) and PGE₂ was detected after the challenge with this dose of OVA (Fig. 3). The relative ratios between the total amount of PGE₂ and those of PGF_{2α}, PGD₂, PGI₂ and TXA₂, released after challenge with 10 000 μg of OVA in control lungs, are presented in Fig. 3.

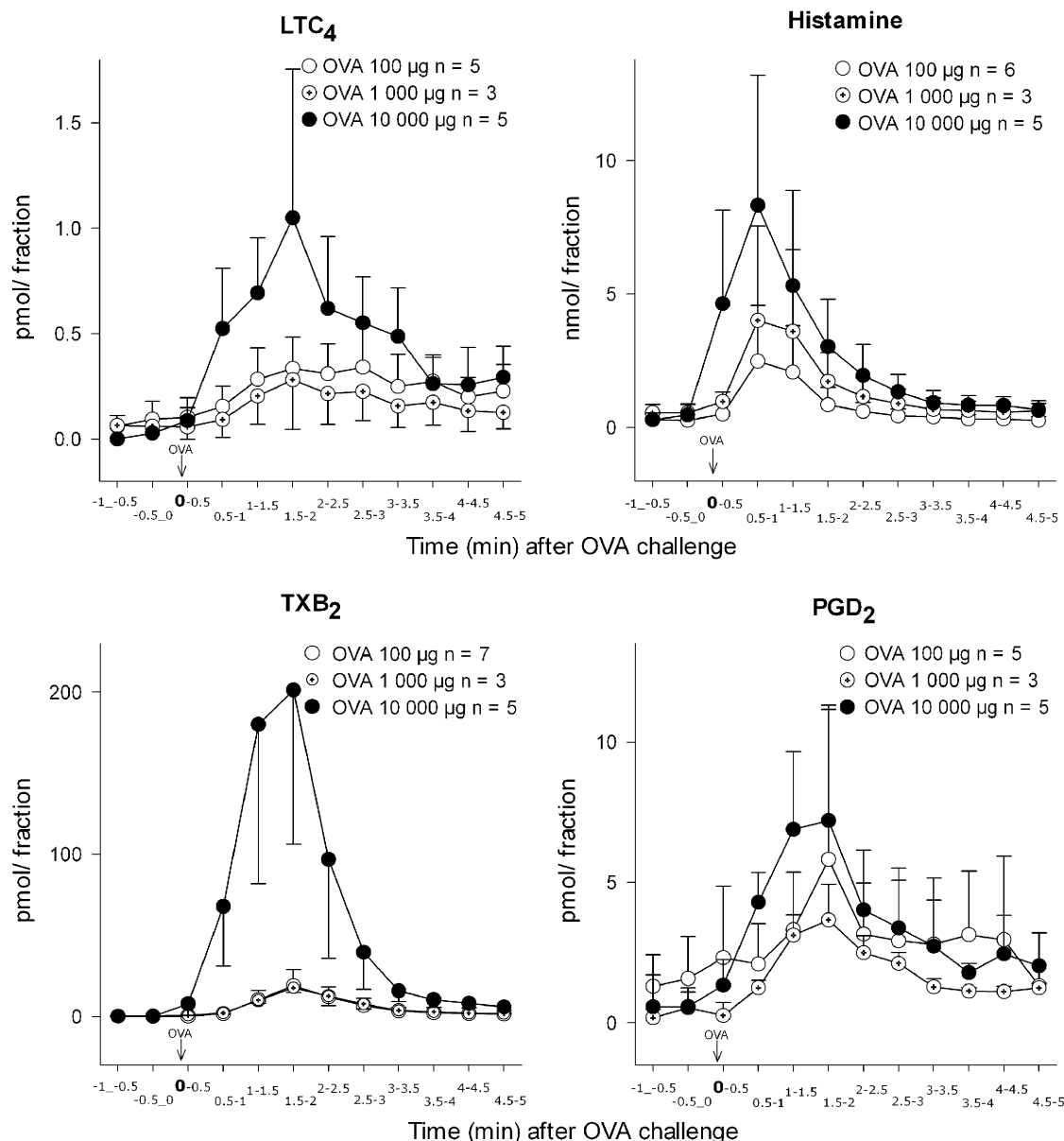


Fig. 1. Dose–response relations for release of LTC₄, histamine, TXA₂ (measured as TXB₂) and PGD₂ after challenge of the lungs from actively sensitised guinea pigs with a single dose of antigen (OVA), 100, 1000 or 10000 µg. Data are presented as the amounts (mean ± SD) of histamine, LTC₄, TXB₂ and PGD₂, measured by enzyme immunoassay in fractions of the lung effluent, collected at baseline (–1 to 0 min) and during 5 min after OVA challenge.

Next, the respective role of the COX-1 and COX-2 isoenzymes as a source for the antigen-induced release of COX products was investigated by interventions with a non-selective COX inhibitor and COX-1 and COX-2 selective inhibitors. The non-selective COX inhibitor flurbiprofen (10 µM) abolished the release of all COX products: TXA₂, PGD₂, PGF_{2α}, PGI₂ and PGE₂ (Fig. 3).

The selective COX-2 inhibitor celecoxib as well as the selective COX-1 inhibitor FR122047 (10 µM each), abolished the release of PGD₂, but neither drug had significant effects on the release of the other COX products (Fig. 3).

3.3. Effects of COX inhibitors on antigen-induced bronchoconstriction

The OVA-induced bronchoconstriction was significantly suppressed by treatment with the non-selective COX inhibitor flurbiprofen (10 µM) (Fig. 4A). In contrast, neither the selective COX-1 inhibitor FR122047 (10 µM) (Fig. 4A) nor the selective COX-2 inhibitor celecoxib (10 µM) (Fig. 4B) had significant inhibitory effects on the dose–effect relation of the OVA-induced bronchoconstriction. It was confirmed that the non-selective COX inhibitor diclofenac (10 µM) also inhibited the antigen-induced bronchoconstriction (Fig. 4B).

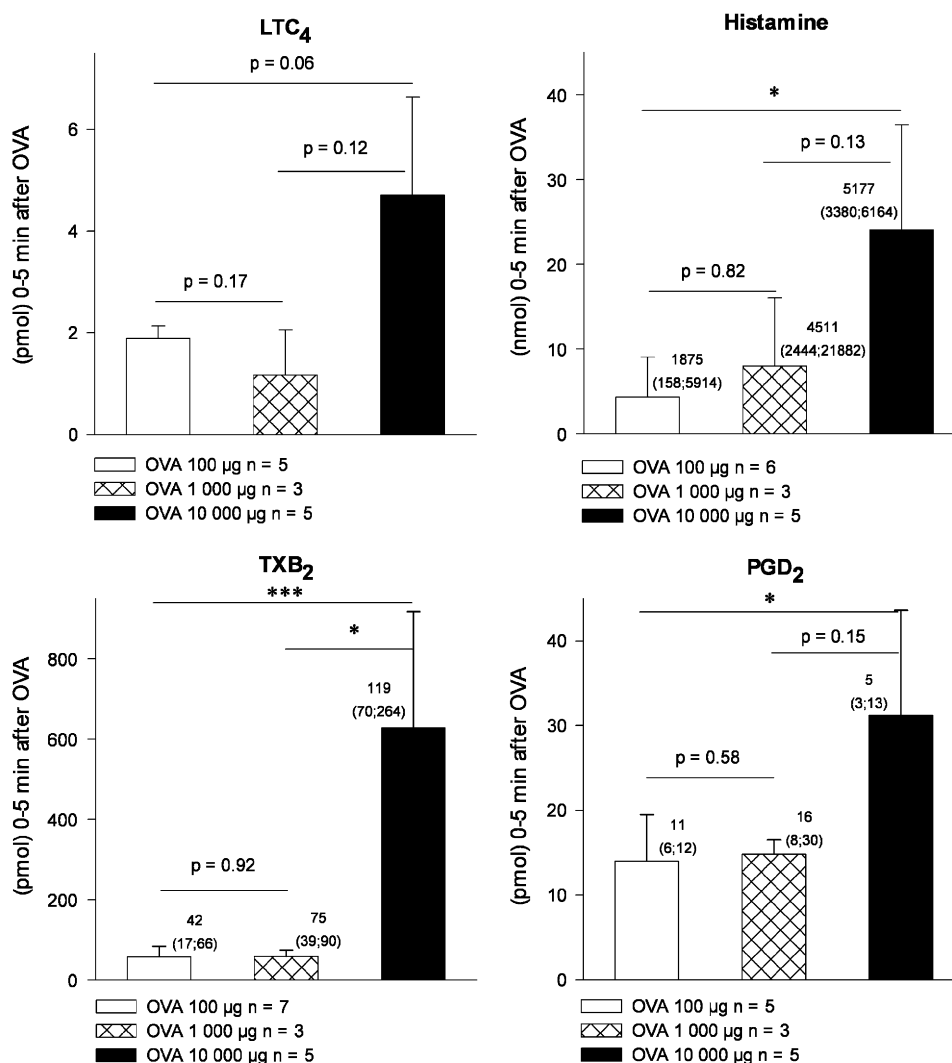


Fig. 2. The total amounts (mean $\pm$ SD) of LTC₄, histamine, TXA₂ (measured as TXB₂) and PGD₂, released during 5 min after the challenge of the lungs from actively sensitised guinea pigs with a single dose of antigen (OVA), 100, 1000 or 10 000 µg. The total amounts of mediators were calculated as area under the curve (AUC): amount (pmol) in the lung effluent vs. time (0–5 min) after OVA challenge. The baseline values 5 min before the challenge were subtracted from the AUC. The relative ratios, expressed as median (min value; max value), between the total amounts of PGD₂, TXA₂ and histamine, and that of LTC₄, released in response to the challenge with three different OVA doses are given in graphs. Level of significance compared with control: * $p < 0.05$, *** $p < 0.001$; SD, standard deviation.

4. Discussion

The primary aim of the present study, performed in the antigen (OVA)-challenged IPL from actively sensitised guinea pigs, was to evaluate the specific role of COX-1 and COX-2 in the immediate antigen-induced generation of prostanoids.

First, the evidence for a role of COX products as mediators of the antigen-induced bronchoconstriction in IPL was confirmed (diclofenac, Fig. 4B) and extended (flurbiprofen, Fig. 4A) in this study by the clear-cut inhibitory effects of these two non-selective COX inhibitors on the airway obstruction. Second, it was established that antigen challenge released the constrictive prostanoids, TXA₂ (measured as immunoreactive TXB₂) and PGD₂ (Figs. 1 and 2). The extended analysis

of COX products released in response to the challenge with the highest antigen dose revealed that in addition to TXA₂ and PGD₂, also PGF_{2 α} was released (Fig. 3). Release of potentially bronchorelaxant prostanoids, such as PGI₂ (measured as immunoreactive 6-keto PGF_{1 α}) and PGE₂, was also detected (Fig. 3). Furthermore, it was established that release of all constrictive prostanoids, TXA₂, PGD₂ and PGF_{2 α} , was abolished by non-selective COX inhibition with flurbiprofen (Fig. 3). This treatment, as well as another non-selective COX inhibitor, diclofenac, also produced significant reduction of the airway obstruction induced by antigen challenge (Figs. 4A, B).

In contrast, it was found that selective COX-1 inhibition as well as selective COX-2 inhibition abolished release of PGD₂, whereas neither treatment had

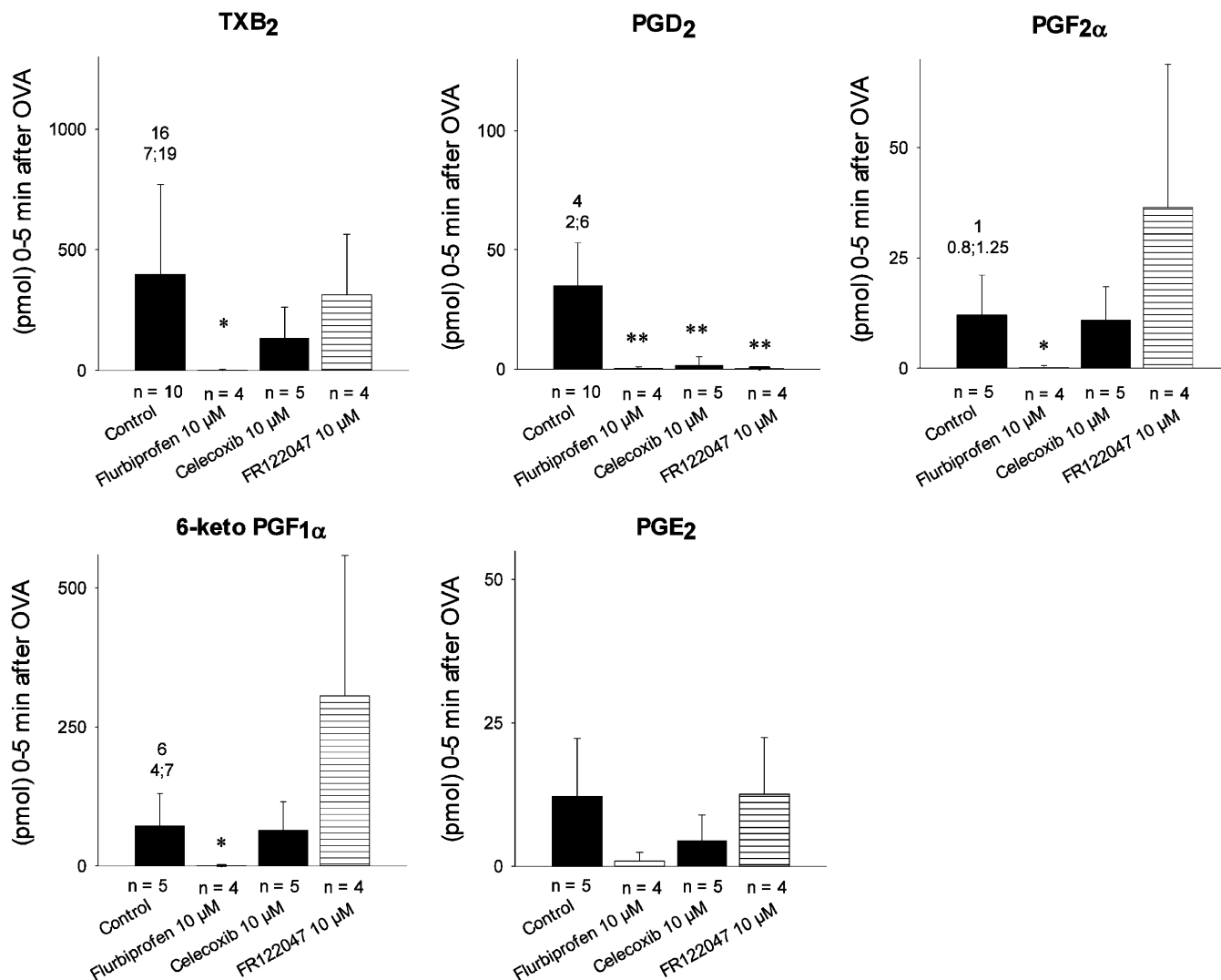


Fig. 3. The total amounts (mean $\pm$ SD) of TXA₂ (measured as TXB₂), PGD₂, PGF_{2 α} , PGI₂ (measured as 6-keto PGF_{1 α}) and PGE₂, released during 5 min after the challenge of the lungs from actively sensitised guinea pigs with a single dose of antigen (OVA), 10 000 μ g, in control or after pretreatment with COX inhibitors: the COX non-selective flurbiprofen, the COX-2 selective celecoxib and the COX-1 selective FR122047 (10 μ M each). The total amounts of prostanoids were calculated as area under the curve (AUC): amount (pmol) in the lung effluent vs. time (0–5 min) after OVA challenge. The baseline values 5 min before the challenge were subtracted from the AUC. The relative ratios, expressed as median (min value; max value), between the total amounts of PGF_{2 α} , PGD₂, PGI₂ and TXA₂, and that of PGE₂, released in response to the control OVA challenge are given in graphs. Level of significance compared with control: * p < 0.05; ** p < 0.01; SD, standard deviation.

significant effects on release of the two other constrictive prostanoids, TXA₂ and PGF_{2 α} (Fig. 3), and the airway obstruction (Figs. 4A, B). We interpret these findings as support for the contribution of both COX-1 and COX-2 to the immediate antigen-induced generation of prostanoids. Our data are thus at variance with observations in cellular *in vitro* models, where the immediate generation of prostanoids utilised COX-1, whereas the delayed prostanoid biosynthesis, induced by pro-inflammatory stimuli, utilised COX-2 [18–21]. One possible implication of our findings might be also that PGH₂ can be used as a substrate by the terminal prostaglandin synthases irrespective of whether generated in reaction initiated by COX-1 or COX-2, at variance with the proposed

selective functional linkage between the two COX isoenzymes and the different terminal prostaglandin synthases [22].

The finding in the present study that only release of PGD₂ was abolished by selective COX-1 and COX-2 inhibition was surprising. However, the substrate affinity of the hematopoietic prostaglandin D synthase (H-PGDS) [23,24] is one order of magnitude lower, as compared to that of the thromboxane synthase [25], the cytosolic lung-type prostaglandin F synthase [26], the cytosolic prostaglandin E synthase [27] and the prostacyclin synthase [28]. It is thus possible that in IPL, the amount of PGH₂ that was available under conditions when one of the two COX isoenzymes was

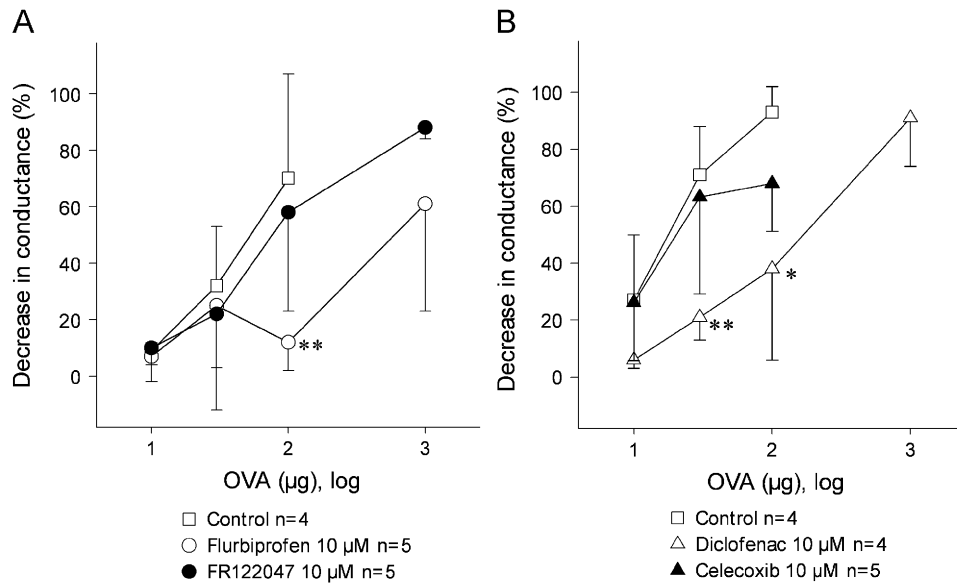


Fig. 4. Effects of COX inhibitors on antigen-induced bronchoconstriction in the lungs from actively sensitised guinea pigs. (A) Dose–response relations for the effect of the challenge with increasing doses of antigen (OVA), performed in control and after pretreatment with the non-selective COX inhibitor flurbiprofen, or the COX-1 selective inhibitor FR122047 (10 µM each) (B) Dose–response relations for the effect of the challenge with increasing doses of antigen (OVA), performed in control and after pretreatment with the non-selective COX inhibitor diclofenac, or the COX-2 selective inhibitor celecoxib (10 µM each). Data are presented as peak effect on airway conductance, expressed as % decrease related to baseline (mean ± SD). Level of significance compared with control: * $p < 0.05$, ** $p < 0.01$; SD, standard deviation.

	Control	COX-1 inhibition	COX-2 inhibition	COX-1/COX-2 inhibition
PGH ₂	+++	+	+	–
PGD ₂	+	–	–	–
PGE ₂	+	+	+	–
PGF _{2α}	+	+	+	–
PGI ₂	++	++	++	–
TXA ₂	+++	+++	+++	–

Fig. 5. Semiquantitative (number of plus) attempt to explain the effects of COX inhibition on the immediate antigen-induced generation of prostanoids in IPL. In control, both COX isoenzymes provide the substrate (PGH₂) to the terminal PG synthases. Treatment with either the selective COX-1 inhibitor or the selective COX-2 inhibitor results in diminished formation of PGH₂. Due to dependency on higher PGH₂ level for H-PGDS [23,24] the selective inhibitors will inhibit PGD₂, but not other prostanoids.

inhibited was not sufficient for generation of PGD₂ by H-PGDS (Fig. 5).

The present study demonstrated also that TXA₂ was the most abundant prostanoid released by the antigen challenge in IPL (Fig. 3), and we have shown previously that on a molar basis, the stable TXA₂ analogue U-46619 was a 100-fold more potent bronchoconstrictor than histamine in this model [16]. It is thus likely that the COX-dependent component in the antigen-induced

bronchoconstriction in IPL is predominantly mediated by TXA₂. This is supported by the finding that the selective COX-1 or COX-2 inhibitor that abolished PGD₂ release had no effect on the antigen-induced bronchoconstriction. This shows that the action of TXA₂, together with that of PGF_{2α}, sufficiently contributed to the airway obstruction. Significant contribution of PGF_{2α} to the antigen-induced bronchoconstriction in this model is however not very likely. This study disclosed that much lower amounts of PGF_{2α} were released by the antigen challenge, as compared to those of TXA₂ (Fig. 3), and we have previously shown that on a molar basis, the bronchoconstrictor potency of PGF_{2α} was 10-fold lower, as compared to that of histamine in this model [36]. In addition, as PGF_{2α} is able to exert the bronchoconstrictive effect in airways also by activation of the prostanoid FP receptor [29], theoretically it is possible that the FP receptor-mediated effect of this prostanoid contribute to the antigen-induced airway obstruction as well. Evaluation of the role of this component is however not possible because to date sufficiently selective FP receptor antagonists are lacking.

The finding in the present study that neither selective COX-1 nor selective COX-2 inhibitor reduced the airway obstruction (Figs. 4A, B) despite suppression of PGD₂ release (Fig. 3), suggests also the minor role of this prostanoid in the antigen-induced bronchoconstriction in this model. This is further supported by the finding that PGD₂ was released by the antigen challenge in much lower amounts, as compared to that of TXA₂ (Fig. 3). The bronchoconstrictor potency of PGD₂, on a

molar basis was equal to that of histamine in this model (Selg et al., submitted manuscript). The specific role of PGD₂ remains to be established by selective inhibition of biosynthesis of this prostanoid.

Taken together, these findings nevertheless suggest the main role of TXA₂ and the minor role of PGF_{2α} and PGD₂ as mediators of the COX-dependent component of the antigen-induced bronchoconstriction in IPL.

The findings in the present study suggest also the predominant role of the constrictive prostanoids and the minor role of the relaxant ones in the immediate antigen-induced bronchoconstriction in IPL, as the observed effects of COX inhibitors on the airway obstruction were apparently dependent only on the effects of these drugs on release of the constrictive prostanoids. The non-selective COX inhibition that abolished release of the constrictive TXA₂, PGD₂ and PGF_{2α} (Fig. 3), was thus associated with significant reduction of the airway obstruction (Figs. 4A, B), in spite of the abolished release also of the bronchoprotective PGE₂ and PGI₂ by this treatment (Fig. 3). The selective COX-1 and COX-2 inhibition, either of which abolished the release of PGD₂, but did not significantly affect that of PGI₂ and PGE₂ (Fig. 4), was not associated with reduction of the airway obstruction (Figs. 1A, B). The bronchoprotective actions of PGE₂ and probably also PGI₂ were thus not sufficient to counteract the bronchoconstrictive effects of TXA₂ and PGF_{2α}. As the effects of COX inhibitors on the EAR are concerned, these drugs were reported to significantly inhibit [10] or do not affect the EAR [30–32]. These conflicting results suggest that constrictive prostanoids contribute to the EAR, but also point to the important role of bronchoprotective prostanoids, such as PGE₂ and PGI₂, which are also inhibited by COX inhibitors in asthmatic airways.

It is likely that prostanoids as well as histamine and CysLTs, detected in this study (Figs. 1 and 2), are predominantly released from mast cells, as a primary effect of the antigen–antibody reaction. However, secondary release of prostanoids due to the specific receptor-mediated effects of the mediators on airway smooth muscle may contribute as well. In the guinea pig perfused lung, histamine liberated TXA₂ [33–35] but not prostaglandins [33]. We have previously detected release of prostanoids by CysLTs in IPL (Selg et al., submitted manuscript), and it remains to define the relative role of primary antigen-induced release of prostanoids from inflammatory cells and secondary mediator-induced release of prostanoids from other effector cells such as smooth muscle.

In summary, the present study confirms the role of COX products as mediators of one component in the immediate antigen-induced bronchoconstriction in IPL. The findings in this study suggest a main role of TXA₂ and a minor role of PGF_{2α} and PGD₂ as mediators of the COX-dependent component in the antigen-induced

bronchoconstriction in IPL. The relative role of these prostanoids however remains to be established. The findings in this study suggest that both COX-1 and COX-2 pathways are involved in the immediate antigen-induced generation of the specific prostanoid end products. It appears that there is no division of labour between COX-1 and COX-2 in the prostanoid-mediated component of antigen-induced bronchoconstriction.

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