



Pulmonary, gastrointestinal and urogenital pharmacology

Antagonising EP₁ and EP₂ receptors reveal that the TP receptor mediates a component of antigen-induced contraction of the guinea pig trachea

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ABSTRACT

The role of different cyclooxygenase (COX) metabolites released after antigen exposure has been difficult to assess due to simultaneous release of dominant constrictors such as histamine and cysteinyl-leukotrienes (CysLT). In addition prostaglandin E₂ (PGE₂) also has a powerful effect on basal tone. The aim was to exclude PGE₂, histamine and CysLTs from the antigen-induced contraction to define the possible involvement of remaining COX metabolites. Isometric force was measured in guinea pig trachea after exposure to cumulatively increasing concentrations of ovalbumin (OVA; 0.1 ng/ml to 0.1 mg/ml) in the absence or presence of biosynthesis inhibitors and receptor antagonists. Challenge with OVA induced a concentration-dependent contraction that reached 75% of maximal tissue response. COX-inhibition or a combination of EP₁ and EP₂ receptor antagonism (ONO-8130 and PF-04418948) completely abolished the tone, resulting in an augmented antigen response. COX inhibition in combination with either antihistamines or antileukotrienes (FLAP inhibitor or CysLT₁₋₂ receptor antagonist) displayed no difference compared to COX inhibition alone. However, a combination of all three classes reduced the contraction to 30%, revealing an unknown contractile component. Exchanging COX inhibition with EP₁₋₂ receptor antagonists together with antihistamines and antileukotrienes could not decrease the contraction more than to 50%. However, when a TP receptor antagonist (SQ-29,548) was further included, the maximal antigen contraction reached 30%, similar as previously, clearly revealing a TP-mediated contractile component. PGE₂ primarily regulate the basal tone via EP₁ and EP₂, whereas prostanoids, such as TXA₂ and PGD₂, contribute as mediator of the antigen-response by activation of the TP receptor.

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1. Introduction

The early allergic reaction displays as an acute bronchoconstriction in asthmatic subjects minutes after antigen exposure (Boulet et al., 2007). In humans and guinea pigs, the predominant mediators responsible for airway smooth muscle contraction are histamine in combination with the lipid mediators cysteinyl-leukotrienes (CysLTs) and cyclooxygenase (COX) metabolites (Björck and Dahlen, 1993; Featherstone et al., 1990; Roquet et al., 1997; Selg et al., 2008). In addition to being potent bronchoconstrictors, these mediators also recruit inflammatory cells, induce mucus secretion and cause vasodilatation, factors aggravating the

airflow obstruction (Bradding et al., 2006; Jutel et al., 2005; Matsuoka et al., 2000).

Because several potent mediators are released concomitantly during the antigen-challenge, single or dual inhibition of the involved mediators has displayed limited effects in several guinea pig *ex vivo* models of antigen-induced airway contraction; such as parenchymal strips (Jonsson and Dahlen, 1994), isolated trachea (Bozkurt et al., 2011), precision cut lung slices (Ressmeyer et al., 2006) and the isolated, perfused and ventilated lung (Sundstrom et al., 2003). However, combining antagonism of histamine receptors with inhibitors of COX and the 5-lipoxygenase pathways inhibits the major part of the antigen induced contraction in several guinea pig models (Riley et al., 2013; Sundstrom et al., 2003). Still, the precise roles and the interactions between the different mediators and their respective receptors have not been established. It remains to determine the relative contributions of the different mediators that have been implicated in the antigen-induced airway constriction. In particular, most previous studies have focused on the role of cysteinyl-leukotrienes and only studied

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the residual component of the contraction after pre-treatment with antihistamines and different non-steroidal anti-inflammatory drugs (NSAIDs) globally blocking formation of all COX products.

Hence, this study aims for the first time to characterise the relative involvement of the individual COX-products under conditions when components in the antigen-induced contraction was defined by step-wise addition of selective inhibitors or antagonists for the main mediators involved. In guinea pig trachea (GPT), this has been particularly difficult due to a constitutive smooth muscle tone maintained by prostaglandin (PG) E₂ that induce a contraction through activation of EP₁ receptors as well as simultaneously evoke relaxation through action by EP₂ receptors (S  fholm et al., 2013). Using both selective EP₁ and EP₂ receptor antagonists, the role for the basal airway smooth muscle tension could, for the first time, be assessed in relation to the antigen-induced contraction and thereby also delineate the role of other prostanoid receptors.

2. Materials and methods

The study was approved by the Swedish animal experimentation ethical review board N257/09 and N323/11.

2.1. Sensitization procedure and tissue preparation

Male albino guinea-pigs (Dunkin-Hartley; 400–450 g; Venray, The Netherlands) were sensitised by a single peritoneal injection containing 100 µg ovalbumin (OVA) grade V and 0.1 g aluminium hydroxide dissolved in 0.8 ml sterile PBS. Animals were killed 14–28 days later (Jonsson and Dahlen, 1994) by an overdose of sodium pentobarbital (Apoteket AB, Stockholm, Sweden) followed by the heart–lung-package rapidly removed and placed in ice-cold Krebs–Ringer PSS buffer (NaCl [118.5 mM], KCl [4.7 mM], KH₂PO₄ [1.2 mM], MgCl₂ [1.2 mM], EDTA [0.03 mM], NaHCO₃ [25 mM], glucose [11.1 mM], and CaCl₂ [2.5 mM] dissolved in demineralised water). The trachea was gently dissected from the surrounding connective tissue, cut into eight intact rings of equal length containing between two and three cartilage rings each and placed in a 5 ml tissue organ bath containing Krebs–Ringer PSS and kept at 37 °C, constantly bubbled with carbogen (5% CO₂ in O₂) to maintain pH at 7.4.

2.2. Experimental procedure

Changes in smooth muscle force were detected using an isometric force-displacement transducer linked to a Grass polygraph. The response was displayed using LabChart 7.0.3 (ADInstruments, Australia). During an equilibration period of 60 min with washes every 15 min, the resting force was mechanically adjusted to 30 mN. As a control of guinea pig tracheal reactivity, histamine (0.1 nM to 0.3 mM) was cumulatively added which was followed by a second wash period and a new 45–60 min equilibration period. To study the action of OVA, cumulative concentrations, from 0.1 ng/ml to 0.1 mg/ml, generating concentration-dependent contraction responses were elicited in the absence and presence of pharmacological tools which were given 45 min prior. Only one OVA response was investigated in each preparation and at the end of the experiment, histamine (1 mM), acetylcholine (1 mM) and potassium chloride (60 mM) were added to the organ bath for 30 min which was used as a reference of the maximal contraction. As a reference for the maximal relaxation, a combination of papverine (0.1 mM) and sodium nitroprusside (0.1 mM) were added to the bath and left for 60 min.

2.3. Pharmacological tools

The unselective COX-inhibitor indomethacin, 5-lipoxygenase (5-LOX) inhibitor zileuton, and 5-LOX activating protein (FLAP) inhibitor MK-886 were used to investigate the involvement of the enzymes participating in producing the PGs and CysLTs. Furthermore, selective receptor antagonists for EP₁ (ONO-8130), EP₂ (PF-04418948), TP (SQ-29,548), H₁ (mepyramine), H₂ (metiamide), H₃ (ciproxifan), H₄ (JNJ7777120), CysLT₁ (montelukast), CysLT₁₋₂ (BAY-u9773), M₁₋₅ (atropine), 5-HT_{2A} (ketanserin) and B₂ (HOE-140) receptors were used to assess respective involvement during the antigen induced contraction.

2.4. Drugs and suppliers

NaCl, KCl, CaCl₂, MgCl₂, NaHCO₃, KH₂PO₄ and glucose were obtained from VWR (West Chester, PA, U.S.A.). Ovalbumin (grade V), histamine dihydrochloride, DMSO, EDTA, metiamide, acetylcholine, indomethacin, ciproxifan, ketanserin, atropine, papverine and sodium nitroprusside were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). SQ-29,548, montelukast, BAY-u9773 were obtained from Cayman Chemical (Ann Arbor, MI, U.S.A.). Zileuton, MK-886, mepyramine, JNJ 7777120 and HOE-140 were purchased from Tocris (Ellisville, MO, U.S.A.). ONO-8130 was a kind gift from ONO Pharmaceuticals (Osaka, Japan). PF-04418948 was a kind gift from Pfizer Central Research Division (Groton, CT, U.S.A.). Stock solutions of indomethacin, SQ-29,548 and BAY-u9773 were dissolved in 95% ethanol. ONO-8130, PF-04418948, JNJ 7777120, ciproxifan, ketanserin, montelukast, capsaizapine, zileuton, MK-886 were dissolved in DMSO. All stock solutions were stored at –20 °C. The other drugs were dissolved in deionized H₂O on the experimental day. Dilutions of drugs were freshly made from stocks prior to each experiment in reducing concentrations of its solvent.

2.5. Calculations and statistics

The response generated by OVA is expressed as percentage compared to maximal contraction in relation to the total contractile response. All data are presented as mean ± S.E.M. Statistical analysis was performed using One-Way ANOVA followed by the Bonferroni's Multiple Comparison Test. For the analysis GraphPad Prism 5.01 (GraphPad Software Inc., San Diego, CA, U.S.A.) was used.

3. Results

3.1. PGE₂ modulates the response to antigen challenge via effects of EP₁ and EP₂ receptors on the basal tone

Following the initial resting period, washes and standard assessment of histamine responsiveness, tracheal segments developed a spontaneous contractile tone that stabilised within 30 min. In sensitised but otherwise untreated controls, the tonus reached a level of 33.7 ± 5.5% in relation to maximal tissue contractile response. Treatment with an unselective COX inhibitor (indomethacin), an EP₁ receptor antagonist (ONO-8130) or a combination of EP₁ and EP₂ receptor antagonists (PF-04418948) completely abolished the tone ($P < 0.05$; Fig. 1A and B) whereas single treatment with the EP₂ receptor antagonist, in contrast, increased the basal tone to 67.1 ± 3.9% ($P < 0.05$; Fig. 1B). Antagonising the TP receptor (SQ-29,548), H₁₋₂ receptors (mepyramine and metiamide), inhibiting the FLAP enzyme (MK-886) or combinations of these components did not affect the initial smooth muscle tone (Table 1). In contrast, zileuton which is considered

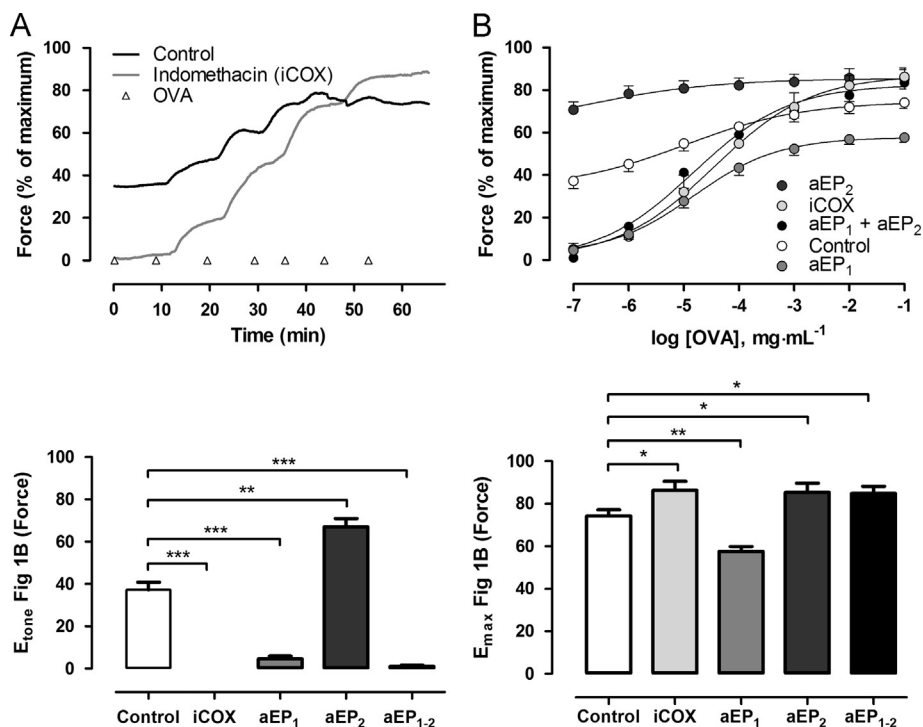


Fig. 1. (A) Representative trace over time after cumulative administration of OVA (0.1 ng/ml to 0.1 mg/ml) in guinea pig trachea, in the presence (control) or absence of indomethacin (iCOX). (B) Concentration-response curves to OVA in the presence of the COX inhibitor indomethacin (iCOX; 3 μ M), EP₁ receptor antagonist ONO-8130 (aEP₁; 10 nM) and EP₂ receptor antagonist PF-04418948 (aEP₂; 10 μ M). The contraction of each segment in all experiments was calculated as percentage of maximal contraction of 1 mM histamine, 1 mM acetylcholine and 60 mM KCl in relation to maximal relaxation, a combination of 0.1 mM papverine and 0.1 mM sodium nitroprusside. Data represent mean $\pm$ S.E.M. ($n=8-23$) and * P -value < 0.05, ** P -value < 0.01, and *** P -value < 0.001.

Table 1
 E_{tone} , E_{max} and pEC_{50} calculated from a non-linear regression fit based on concentration-response curve to ovalbumin in isolated guinea pig trachea.

Antagonist/inhibitor target	E_{tone}	E_{max}	pEC_{50}	n
Control	33.7 $\pm$ 5.5	74.6 $\pm$ 2.7	5.0 $\pm$ 0.4	23
aTP	41.3 $\pm$ 6.0	72.5 $\pm$ 3.7	5.1 $\pm$ 0.5	5
aTP + aH ₁₋₂	32.7 $\pm$ 6.0	73.8 $\pm$ 3.1	5.0 $\pm$ 0.5	5
aTP + iFLAP	40.9 $\pm$ 5.2	72.6 $\pm$ 4.6	4.2 $\pm$ 0.6	7
iFLAP	45.4 $\pm$ 5.0	64.8 $\pm$ 2.7	5.3 $\pm$ 0.7	8
iFLAP + aH ₁₋₂	36.1 $\pm$ 12.8	66.1 $\pm$ 5.9	5.1 $\pm$ 2.0	6
aH ₁₋₂	46.3 $\pm$ 5.1	68.8 $\pm$ 3.3	5.1 $\pm$ 0.6	5
i5-LO	6.8 $\pm$ 3.6 ^a	80.6 $\pm$ 2.7	4.6 $\pm$ 0.2	5

TP receptor antagonist SQ-29,548 (aTP; 1 μ M), H₁ receptor antagonist mepyramine (aH₁; 1 μ M), H₂ receptor antagonist metiamide (aH₂; 1 μ M), FLAP inhibitor MK-886 (iFLAP; 10 μ M) and 5-LO inhibitor zileuton (i5-LO; 1 μ M). The contraction of each segment in all experiments was calculated as percentage of maximal contraction of 1 mM histamine, 1 mM acetylcholine and 60 mM KCl in relation to maximal relaxation to a combination of 0.1 mM papverine and 0.1 mM sodium nitroprusside. Data represent mean $\pm$ S.E.M.

^a P -value < 0.05.

to inhibit 5-LO enzyme caused an almost complete abolishment of the tone ($P < 0.05$).

Cumulative administration of OVA (0.1 ng/ml to 0.1 mg/ml) induced reproducible concentration-dependent contractions both in presence and absence of COX inhibition. However, in the presence of COX inhibition the maximal contractile force to OVA was significantly greater ($87.1 \pm 3.9\%$; $P < 0.05$) compared to sensitised untreated controls ($74.6 \pm 2.7\%$). Treatment with ONO-8130 significantly decreased the response ($57.7 \pm 1.6\%$; $P < 0.05$), whereas PF-04418948, alone or in combination with ONO-8130, increased the response to a similar level as COX inhibition ($86.1 \pm 4.5\%$ and $84.8 \pm 3.2\%$, respectively; $P < 0.05$; Fig. 1A and B). On the contrary, in absence of COX inhibition, maximal OVA response was

not affected by single or combined treatment with SQ-29,548, mepyramine, metiamide and MK-886 (Table 1).

3.2. Antagonising the EP₁ and the EP₂ receptors unmask an antigen driven TP receptor contraction

In order to investigate the relative contribution of prostanoid receptors for the contraction, the influence of the constitutively produced PGE₂ was excluded by either antagonising both the EP₁ and EP₂ receptors or EP₁ receptor only. Further, since the strong contractions to histamine and CysLTs, which are known to be released by mast cells (Adams and Lichtenstein, 1979), can conceal the prostanoid effects, these experiments were also performed in presence of antagonists for H₁₋₂ and CysLT₁₋₂ receptors (BAY-u9773). In addition, as PGD₂ and TXA₂, also released by mast cells, both cause contraction in guinea pig lung through TP receptors (Featherstone et al., 1990), this receptor was also antagonised. Thus, when EP₁₋₂, H₁₋₂, CysLT₁₋₂ and TP receptors were antagonised the antigen-induced contraction reached an E_{max} of $28.7 \pm 0.7\%$ that was significantly lower than using only EP₁₋₂ receptor antagonists ($P < 0.05$; Fig. 2A). Keeping the similar set-up however exchanging BAY-u9773 with the FLAP-inhibitor MK-886 gave an identical E_{max} ($28.0 \pm 2.7\%$). Distinctly, when the TP receptor antagonist was excluded, a marked increase of the E_{max} was obtained ($47.7 \pm 6.2\%$; $P < 0.05$). The TP receptor component was also demonstrated in experiments when the EP₂ receptor antagonist was omitted. In these experiments the contractions with all other antagonists used were totally abolished regardless if BAY-u9773 or MK-886 was used to block the CysLT component (Fig. 2B). Yet, when the TP receptor antagonist was excluded a marked response was obtained with an E_{max} of $24.7 \pm 3.4\%$ ($P < 0.05$). The different combinations of receptor antagonists and enzyme inhibitors did neither affect maximal contraction nor maximal relaxation.

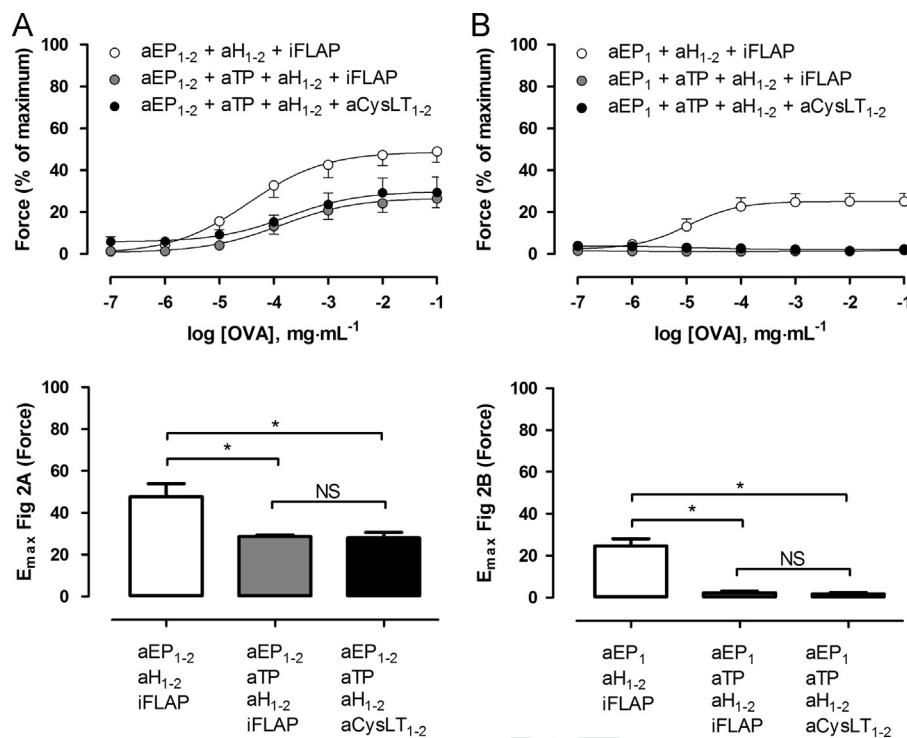


Fig. 2. (A) and (B) Concentration-response curves to OVA in the presence of EP₁ receptor antagonist ONO-8130 (aEP₁; 10 nM) and EP₂ receptor antagonist PF-04418948 (aEP₂; 10 μM), TP receptor antagonist SQ-29,548 (aTP; 1 μM), H₁ receptor antagonist mepyramine (aH₁; 1 μM), H₂ receptor antagonist metiamide (aH₂; 1 μM), FLAP enzyme inhibitor MK-886 (iFLAP; 10 μM) and CysLT₁₋₂ receptor antagonist BAY-u9773 (aCysLT₁₋₂; 1 μM). The contraction of each segment in all experiments was calculated as percentage of maximal contraction of 1 mM histamine, 1 mM acetylcholine and 60 mM KCl in relation to maximal relaxation, a combination of 0.1 mM papverine and 0.1 mM sodium nitroprusside. Data represent mean ± S.E.M. ($n=6-10$) and * P -value < 0.05, NS=non-significant at $\alpha=0.05$.

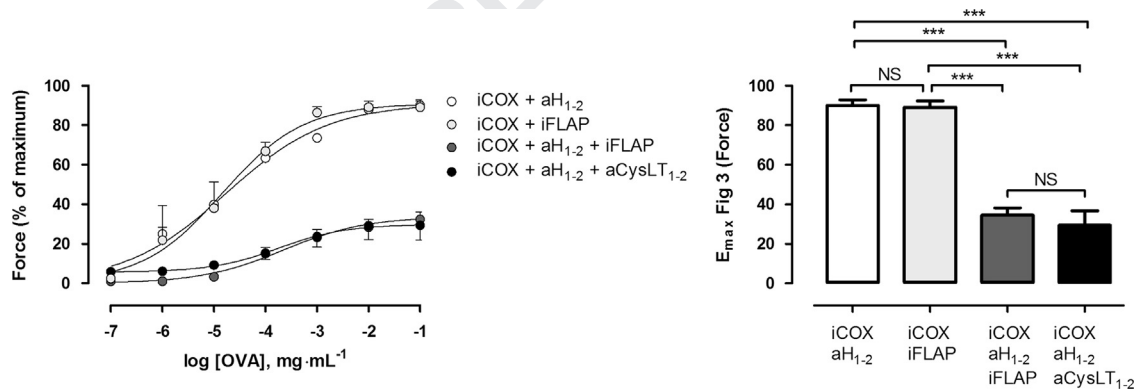


Fig. 3. Concentration-response curves to OVA in the presence of the COX inhibitor indomethacin (iCOX; 3 μM), H₁ receptor antagonist mepyramine (aH₁; 1 μM), H₂ receptor antagonist metiamide (aH₂; 1 μM), CysLT₁₋₂ receptor antagonist BAY-u9773 (aCysLT₁₋₂; 1 μM) and FLAP enzyme inhibitor MK-886 (iFLAP; 10 μM). The contraction of each segment in all experiments was calculated as percentage of maximal contraction of 1 mM histamine, 1 mM acetylcholine and 60 mM KCl in relation to maximal relaxation, a combination of 0.1 mM papverine and 0.1 mM sodium nitroprusside. Data represent mean ± S.E.M. ($n=5-12$) and *** P -value < 0.001, NS=non-significant at $\alpha=0.05$.

3.3. Inhibition of histamine and cysteinyl-leukotrienes in the presence of COX inhibition reduces antigen-induced contraction and unmasks an unknown residual contractile component

COX inhibition in combination with either H₁₋₂ antagonism or FLAP inhibition displayed no difference in contraction as compared to COX inhibition alone. However, segments treated with a combination consisting of COX inhibition, H₁₋₂ receptor antagonists and FLAP inhibitor clearly displayed a reduced maximal OVA-induced contraction, reaching an E_{max} of $32.4 \pm 3.6\%$ ($P < 0.05$; Fig. 3). When the FLAP inhibitor was exchanged for a CysLT₁₋₂ receptor antagonist, a similar inhibition was observed, reaching an E_{max} of $29.6 \pm 3.3\%$ ($P < 0.05$). However, when the COX inhibition

and antihistamines were used in combination with the CysLT₁ receptor antagonist montelukast, the reduction was absent, reaching an E_{max} of $79.5 \pm 2.4\%$.

The residual response, that was observed regardless if the segments were treated with either EP₁₋₂ and TP receptor antagonists or COX enzyme inhibition in combination with H₁₋₂ receptor antagonists and FLAP inhibition, was further investigated by further addition of antagonists for either the H₃ and the H₄ receptors (ciproxifan and JNJ 7777120, respectively), M₁₋₅ receptors (atropine), 5-HT_{2A} receptor (ketanserin) or B₂ receptor (HOE-140). Still, all combinations were found to be ineffective in reducing the remaining residual component unmasked in the antigen-induced contraction (Table 2).

Table 2

E_{tone} , E_{max} , and pEC_{50} calculated from a non-linear regression fit based on concentration-response curve to ovalbumin in isolated guinea pig trachea.

Antagonist/inhibitor target	E_{tone}	E_{max}	pEC_{50}	n
iCOX + aH ₁₋₂ + iFLAP	1.7 ± 0.8	38.0 ± 2.9	3.6 ± 0.3	14
iCOX + aH ₁₋₄ + iFLAP	0.7 ± 0.4	41.2 ± 2.9	3.8 ± 0.2	7
iCOX + aH ₁₋₂ + iFLAP + aM ₁₋₅	0.7 ± 0.5	42.3 ± 8.7	3.3 ± 0.7	6
iCOX + aH ₁₋₂ + iFLAP + a5-HT _{2A}	0.1 ± 0.0	37.4 ± 9.0	3.0 ± 0.8	6
iCOX + aH ₁₋₂ + iFLAP + aB ₂	0.1 ± 0.0	32.9 ± 5.2	3.9 ± 0.6	6

COX inhibitor indomethacin (iCOX; 3 μM), H₁ receptor antagonist mepyramine (aH₁; 1 μM), H₂ receptor antagonist metiamide (aH₂; 1 μM), H₃ receptor antagonist ciproxifan (aH₃; 1 μM), H₄ receptor antagonist JNJ7777120 (aH₄; 1 μM), FLAP inhibitor MK-886 (iFLAP; 10 μM), muscarinic M₁₋₅ receptor antagonist atropine (aM₁₋₅; 1 μM), serotonin receptor 2A antagonist ketanserin (a5-HT_{2A}; 100 nM) and bradykinin B₂ receptor antagonist HOE-140 (aB₂; 10 nM). The contraction of each segment in all experiments was calculated as percentage of maximal contraction of 1 mM histamine, 1 mM acetylcholine and 60 mM KCl in relation to maximal relaxation to a combination of 0.1 mM papverine and 0.1 mM sodium nitroprusside. Data represent mean ± S.E.M.

4. Discussion

Analysis of the antigen-induced contraction in guinea pig trachea is confounded by the powerful airway smooth muscle tone maintained by PGE₂ acting on contractile EP₁ receptors and counteracted by relaxant EP₂ receptors (S  fholm et al., 2013). However, with pharmacological interventions using selective receptor antagonists and enzyme inhibitors we have shown that the airway smooth muscle contraction induced by antigen is essentially mediated by the combined action of histamine, cysteinyl-leukotrienes and prostanoids that act on TP receptors. After complete inhibition of these three targets, only a small residual constriction component remained. This study shows that the airway smooth muscle antigen-induced response in guinea pig closely mimics the early allergic reaction displayed in isolated human bronchi (Bjorck and Dahlen, 1993) or in asthmatic subjects after antigen exposure (Roquet et al., 1997). Clearly, to achieve a strong inhibition of the antigen response, several receptors needs to be blocked simultaneously.

It has previously been reported that removing endogenously produced prostanoids, using NSAIDs, completely abolish the basal airway tone of the guinea pig trachea (Orehek et al., 1973). This inhibition has been shown to be due to reduced levels of endogenous PGE₂ resulting in a loss of EP₁ receptor-mediated contraction (S  fholm et al., 2013). In the present study, this inhibitory effect was confirmed by both indomethacin and the selective EP₁ receptor antagonist ONO-8130, whereas antagonists for H₁, H₂, or TP receptors or the FLAP inhibitor MK-886 had no effect on the basal tone. On the other hand, when the EP₂ receptor was antagonised, the basal tone was increased, thus confirming the balance between EP₁ receptor mediated contraction and EP₂ receptor mediated relaxation in the isolated guinea pig trachea (S  fholm et al., 2013). However, zileuton, which generally is considered to be a 5-LOX inhibitor also reduced the basal tone. This may be explained by the recent observation that zileuton in higher concentration also displays COX-inhibition in mouse peritoneal macrophages and in human whole blood (Rossi et al., 2010). Furthermore, the findings that the smooth muscle tone was not reduced by the FLAP enzyme inhibitor MK-886, the unselective CysLT receptor antagonist L-648,051 or by BAY-u9773 (Cuthbert et al., 1991; Linden et al., 1991), supports the hypothesis that zileuton also inhibits PGE₂ production in the guinea pig trachea. Thus, due to this additional effect, zileuton was not used further in the combinations.

Cumulative OVA exposure of the isolated trachea from sensitised guinea pigs caused a reproducible antigen-induced contraction

irrespectively of initial smooth muscle tone. Addition of the unselective COX inhibitor, indomethacin amplified the maximum contractile response to the antigen. This augmented response after COX inhibition or unselective EP receptor antagonism AH-6809, seen both in human bronchi (Adams and Lichtenstein, 1985; Gorenne et al., 1994) and guinea pig trachea (Burka and Paterson, 1980; Hand and Buckner, 1979), has been hypothesised to be caused by enhanced release of histamine by removal of inhibitory cyclooxygenase products, such as PGE₂ (Undem et al., 1987). Indeed, when the EP₂ receptor was blocked, the maximum contraction was increased both with and without EP₁ receptor blockade, confirming that the increased response was due to inhibition of PGE₂. It is not possible to conclude from these experiments whether this is caused by reduced release of histamine, inhibition of the relaxation, or a combination. However, we have demonstrated a role for the contractile EP₁ receptor by the reduced maximum effect of the antigen when this receptor was blocked.

To rule out a further prostanoid component in the contraction, experiments were performed comparing the effect of general COX-inhibition with selective blockade of PGE₂. The latter was assessed by selectively blocking EP₁ and EP₂ receptors which have been shown to be the EP receptors responsible for the contractile and relaxant effect of PGE₂ in guinea pig trachea, respectively (S  fholm et al., 2013). However, to isolate this component the effect of both histamine and CysLTs also had to be inhibited. Thus, in the presence of H₁₋₂ antagonism and FLAP inhibition with the tone removed by either an EP₁ receptor antagonist or both EP₁ and EP₂ receptor antagonists, a contraction approximately 20% greater was obtained compared to when prostanoid synthesis were inhibited. This effect was abolished by the TP receptor antagonist SQ-29,548. It is established that TXA₂ and PGD₂ are released during antigen-induced degranulation (Turner and Dollery, 1988) exerting their main contractile effect in the lung through the TP receptor (Beasley et al., 1989; Larsson et al., 2011). Thus, after removal of the tone in combination with antihistamines and FLAP inhibition, the contribution of the TP component could be identified.

As described previously, in both human and guinea pig, the main components of the antigen-induced contraction are mediated by histamine and CysLTs (Bjorck and Dahlen, 1993; Burka and Paterson, 1981) and combined inhibition of these mediators are needed to achieve substantial inhibition (Sundstrom et al., 2003). Thus, neither blockade of H₁₋₂ receptors or inhibiting the response to CysLTs using the selective FLAP inhibitor MK-886 and nonselective CysLT₁₋₂ receptor antagonist BAY-u9773 affected the maximum antigen response. One possible reason for this phenomenon is that the responses to both agonists are close to the maximum possible response so that any additive effect cannot be distinguished. Alternatively, the receptors may compete for the same intracellular signalling system. Here we have also shown that combined CysLT₁₋₂ receptor antagonism was required, which may be due to the same additive effect.

Irrespectively of whether the antigen-induced contraction was reduced using antihistamines in combination with complete inhibition of COX and 5-LOX metabolites, or by the use of antagonists targeting EP₁₋₂, TP or CysLT₁₋₂ receptors, the outcome was the same: an approximately 70% reduction of the antigen-induced contraction. Furthermore, atropine, ketanserin and HOE-140, all failed to alter this response indicating that muscarinic M₁₋₅ receptors, serotonin 5-HT_{2A} and bradykinin BK-B₂ receptors respectively, do not mediate antigen-induced contraction in the guinea pig trachea.

In conclusion, antigen-induced contractile responses to OVA in the guinea pig central airway appears to be due to a major release of histamine and 5-LOX products together with a significant effect induced by activation of TP receptors so that inhibition of all three classes of receptor is required to substantially reduce the effect.

These new findings are important for several reasons. Firstly, since the specific role of PGE₂ has not been established before, both a selective and potent EP₁ and EP₂ receptor antagonist were used revealing PGE₂ as a powerful modulator of the antigen-induced response. Secondly, by the use of several receptor antagonists, the specific contribution of the TP receptor could for the first time be identified. Thirdly, since the reduction of the antigen-induced contraction was minimal when the effect generated by two mediator classes was inhibited, it was necessary to investigate if inhibition by all three mediator classes, histamine, prostaglandins and leukotrienes, had any effect at reducing the airway smooth muscle contraction. This study increases our understanding of the mediators released in conjunction with mast cell degranulation and has shown that inhibition of three classes of mediator is needed to achieve significant reduction of the antigen-induced contraction. As complete COX inhibition induces contractile amplification in both human and guinea pig (Adams and Lichtenstein, 1985; Bozkurt et al., 2011), an alternative therapeutic approach might be to combine selective receptor antagonists instead of global enzyme inhibition, which risks disrupting any potentially protective receptors such as EP₂ in guinea pig and EP₂ and EP₄ in human (Buckley et al., 2011; S  fholm et al., 2013). From our findings, we conclude that PGE₂ primarily regulates basal tone whereas prostanoids, such as TXA₂ and PGD₂ contribute as mediators of the antigen-response by activation of the TP receptor.

Q2 Uncited reference

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