



# LUND UNIVERSITY

## Prostaglandin D(2) induces contractions through activation of TP receptors in peripheral lung tissue from the guinea pig.

Larsson Callerfelt, Anna-Karin; Hagfjärd, Annika; Dahlén, Sven-Erik; Adner, Mikael

*Published in:*  
European Journal of Pharmacology

*DOI:*  
[10.1016/j.ejphar.2011.07.046](https://doi.org/10.1016/j.ejphar.2011.07.046)

2011

[Link to publication](#)

*Citation for published version (APA):*  
Larsson Callerfelt, A.-K., Hagfjärd, A., Dahlén, S.-E., & Adner, M. (2011). Prostaglandin D(2) induces contractions through activation of TP receptors in peripheral lung tissue from the guinea pig. *European Journal of Pharmacology*, 669(1-3), 136-142. <https://doi.org/10.1016/j.ejphar.2011.07.046>

*Total number of authors:*  
4

### General rights

Unless other specific re-use rights are stated the following general rights apply:  
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

### Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117  
221 00 Lund  
+46 46-222 00 00

Prostaglandin D<sub>2</sub> induces contractions through activation of TP receptors in peripheral lung tissue from the guinea pig

Anna-Karin Larsson<sup>a, b\*</sup>, Annika Hagfjård<sup>a</sup>, Sven-Erik Dahlén<sup>a</sup> and Mikael Adner<sup>a</sup>

<sup>a</sup> Experimental Asthma and Allergy Research, The Institute of Environmental Medicine, Karolinska Institutet, 171 77 Stockholm, <sup>b</sup> Lung Biology, Department of Experimental Medical Science, Lund University, 221 84 Lund, Sweden

*E-mail addresses:*

Anna-Karin.Larsson@med.lu.se

Annika.Hagfjard@gmail.com

Sven-Erik.Dahlen@ki.se

Mikael.Adner@ki.se

*\* Corresponding author:*

Anna-Karin Larsson, Lung Biology, Department of Experimental Medical Science, BMC D12, Lund University, 221 84 Lund, Sweden. Tel: +46 462229441

E-mail: Anna-Karin\_L.Larsson@med.lu.se

1 **Abstract**

2 Prostaglandin D<sub>2</sub> (PGD<sub>2</sub>), released through mast cell activation, is used as a non-invasive  
3 biomarker in patients with asthma. Since PGD<sub>2</sub> can elicit opposing effects on airway tone via  
4 activation of the PGD<sub>2</sub> receptors DP<sub>1</sub> and DP<sub>2</sub> as well as the thromboxane receptor TP, the  
5 aim of this study was to characterize the receptors that are activated by PGD<sub>2</sub> in the guinea  
6 pig lung parenchyma. PGD<sub>2</sub> and the thromboxane analogue U46619 induced concentration-  
7 dependent contractions. U46619 was more potent and caused stronger effect than PGD<sub>2</sub>. The  
8 specific TP receptor antagonist SQ-29548 and the combined TP and DP<sub>2</sub> receptor antagonist  
9 BAYu3405 concentration-dependently shifted the curves for both agonists to the right. The  
10 DP<sub>1</sub> receptor agonist BW245 induced a weak relaxation at high concentrations, whereas the  
11 DP<sub>1</sub> receptor antagonist BWA868C did not affect the PGD<sub>2</sub> induced contractions. The  
12 specific DP<sub>2</sub> receptor agonist 13,14-dihydro-15-keto -PGD<sub>2</sub> showed neither contractile nor  
13 relaxant effect in the parenchyma. Furthermore, studies in precision-cut lung slices specified  
14 that airways as well as pulmonary arteries and veins contracted to both PGD<sub>2</sub> and U46619.  
15 When the lung parenchyma from ovalbumin sensitized guinea pigs were exposed to  
16 ovalbumin, both thromboxane B<sub>2</sub> and PGD<sub>2</sub> were released. Ovalbumin also induced maximal  
17 contractions at similar level as PGD<sub>2</sub> in the parenchyma, which was partly reduced by SQ-  
18 29548. These data show that PGD<sub>2</sub> should be recognized as a TP receptor agonist in the  
19 peripheral lung inducing contraction on airways, arteries and veins. Therefore, a TP receptor  
20 antagonist can be useful in combination treatment of allergic responses in asthma.

21

22

23 **Keywords:** Guinea pig; lung parenchyma; ovalbumin; precision cut lung slices; prostaglandin  
24 D<sub>2</sub>; thromboxane

## 25 **1. Introduction**

26 Prostaglandin D<sub>2</sub> (PGD<sub>2</sub>), secreted during mast cell activation (Dahlen and Kumlin, 2004),  
27 and its metabolite 9 $\alpha$ -11 $\beta$  PGF<sub>2 $\alpha$</sub>  are used as a non-invasive biomarkers in patients with  
28 asthma (O'Sullivan et al., 1996). PGD<sub>2</sub> is part of the acute asthmatic airway response; levels  
29 of this mediator can be found within minutes in BAL fluid and at 150-fold higher biologically  
30 active levels than before the exacerbation (Liu et al., 1991). In addition to the acute reaction,  
31 PGD<sub>2</sub> has through recruitment of inflammatory cells been suggested to contribute to the  
32 formation of the chronic asthmatic inflammation and subsequent airway remodelling (Balzar  
33 et al., 2011).

34

35 PGD<sub>2</sub>, generated from arachidonic acid, is converted to PG via cyclooxygenase (COX) (Vane,  
36 1971) and PGD synthase (Urade and Eguchi, 2002). There are two distinct types of PGDS;  
37 hematopoietic (H-PGDS) and lipocalin-type (L-PGDS). H-PGDS is highly expressed in mast  
38 cells, eosinophils, macrophages, and lymphocytes as well as structural cells such as epithelial  
39 cells and fibroblasts, whereas L-PGDS is mainly expressed in the central nervous system and  
40 heart (Okano et al., 2006). PGD<sub>2</sub> exits the cell via a carrier-mediated process and activates  
41 specific G-protein coupled receptors on target cells. PGD<sub>2</sub> is classified to mediate its effect via  
42 the DP<sub>1</sub> (Coleman et al., 1994) and the DP<sub>2</sub> (CRTH<sub>2</sub>) receptors (Abe et al., 1999), but also  
43 known to act via the receptor for tromboxane A<sub>2</sub> (TXA<sub>2</sub>), the TP receptor (Hamid-Bloomfield  
44 et al., 1990). The DP<sub>1</sub> receptor is widely distributed in airway and vascular smooth muscle,  
45 blood platelets, airway epithelium and nervous tissue (Coleman et al., 1994; Matsuoka et al.,  
46 2000; Norel et al., 1999). PGD<sub>2</sub> has also an important chemotactic role via activation of the  
47 DP<sub>2</sub> receptor (Abe et al., 1999), which is mainly expressed on Th2 cells and eosinophils (Abe  
48 et al., 1999) but also on human airway smooth muscle (Abe et al., 1999; Parameswaran et al.,  
49 2007). The TP receptors are expressed on bronchial and vascular smooth muscle cells, blood

50 platelets and myofibroblasts (Capra et al., 2003; Coleman et al., 1994) and are known to  
51 mediate a strong and long-lasting contraction in these tissues (Held et al., 1999; Ressmeyer et  
52 al., 2006). PGD<sub>2</sub> may thus have broad actions since activation of multiple receptors can elicit  
53 theoretically opposing effects on airway tone.

54

55 Although the lung parenchyma is a complex tissue, the action in the peripheral lung is of  
56 importance to study since asthma is suggested to be a disease of the small airways (van den  
57 Berge et al., 2011). Especially the action of PGD<sub>2</sub> is of interest since it has been shown that  
58 mast cells are located peripherally around small bronchi, vessels and further out to the alveoli  
59 (Andersson et al., 2009) and thus may not only affect airways. The aim of this study was  
60 therefore to characterize the receptors that are activated by PGD<sub>2</sub> in the peripheral lung and  
61 subsequently investigate the significance of this effect in allergen-induced contractions. The  
62 guinea pig parenchyma is particularly suitable as it has been shown to respond to many  
63 agonists similar to human (Canning and Chou, 2008; Ressmeyer et al., 2006).

64 **2. Methods**

65 *2.1. Animals and ovalbumin-sensitization*

66 Male Dunkin Hartley guinea pigs (300–350 g b.w.) were used. In one part of the experiments  
67 the guinea pigs were sensitized to ovalbumin at least four weeks prior to experiments as  
68 previously described (Larsson et al., 2005). The study was approved by the regional  
69 committee of animal experimentation ethics (N127/04, N63/07).

70

71 *2.2. Lung parenchymal strips and organ bath experiments*

72 The animals were sacrificed by an overdose of inhaled CO<sub>2</sub> and the heart-lung-package was  
73 quickly removed and placed in ice-cold Tyrode's solution (prepared each day, containing  
74 NaCl 149.2 mM, KCl 2.7 mM, NaHCO<sub>3</sub> 11.9 mM, glucose 5.5 mM, CaCl<sub>2</sub> 1.8 mM, MgCl<sub>2</sub>  
75 0.5 mM, NaH<sub>2</sub>PO<sub>4</sub> 0.4 mM). The lung parenchyma was cut parallel to the peripheral  
76 margins, yielding four to eight strips, each having a size of 2×2×20 mm and a weight of  
77 approximately 60 mg. The parenchymal strips were set up at a resting tension of 4.0 mN in 5  
78 ml organ baths filled with Tyrode's solution, bubbled with carbogen gas (6.5% CO<sub>2</sub> in O<sub>2</sub>) to  
79 keep a pH of 7.4 at 37°C. Changes in smooth muscle tension, contractions and relaxations,  
80 were recorded via isometric force-displacement transducers connected to a Grass polygraph.  
81 After an equilibration period of 90 min and washes each 15 min, histamine was added as a  
82 control of the parenchymal strip reactivity. Preparations displaying contraction responses less  
83 than 1.0 mN to 30 μM of histamine were excluded from further experiments. Another wash  
84 and equilibration period between histamine and treatment period was performed. All  
85 antagonists were given 15 min before the challenges. For study the effect of different  
86 agonists, the parenchymal strip was exposed to cumulative concentrations. To study the  
87 relaxation the parenchyma was pre-contracted with 10 nM of LTD<sub>4</sub> generating a 50%  
88 contraction. To study the allergic early phase reaction, ovalbumin was added as cumulative

89 challenge of increasing concentrations every 10 min without changing bath fluid. Maximum  
90 contractions of the preparation were determined with histamine (1 mM), acetylcholine (1  
91 mM) and potassium chloride (KCl; 50 mM) at the end of each experiment, and other  
92 responses were expressed as percent of maximum contractions.

93

### 94 *2.3. Measurements of released mediators with enzyme immunoassays*

95 A 1 mL aliquot of organ bath fluid was collected from each organ bath and immediately  
96 frozen at -20°C. The samples were taken at the end of the equilibration period to obtain basal  
97 mediator release from the tissue and at the obtained contractile plateau after challenge with  
98 ovalbumin 1000 ng/ml. Enzyme immunoassay (EIA) analyses of the prostanoids TXA<sub>2</sub> and  
99 PGD<sub>2</sub> were performed according to the manufacturer's instructions. TXA<sub>2</sub> was measured as  
100 the stable metabolite TXB<sub>2</sub>. PGD<sub>2</sub> was measured as PGD<sub>2</sub>-mox. The assay detection limits in  
101 the bath fluid levels were 7.8 pg/ml. The EIA specificity for the different mediators to  
102 interfere with each other was less than 0.01%, with the exception of the EIA kit for TXB<sub>2</sub> that  
103 cross reacted with PGD<sub>2</sub> (0.53%).

104

### 105 *2.4. Precision-cut lung slices*

106 Guinea pig precision-cut lung slices were prepared as previously described (Ressmeyer et al.,  
107 2006). Briefly, the lung was filled through the trachea with a low melting-point agarose  
108 solution (0.75%) containing salbutamol (1 mM). Lung tissue cores were prepared and cut into  
109 220-mm-thick slices with a Krumdieck tissue slicer (Alabama Research and Development,  
110 Munford, AL, USA). Tissue slices were incubated at 37°C in a humid atmosphere in minimal  
111 essential medium supplemented with sodium pyruvate, amino acids, vitamins and glutamine.  
112 The medium was changed on a regular basis during four hours in order to remove the agarose  
113 and cell debris from the tissue. Salbutamol was added to the medium during the first three

114 hours. The slices were then imaged using an analogue (JAI 2040; JAI Pulnix, Alzenau,  
115 Germany) or digital camera (IRB640; Visitron Systems, Munich, Germany). For  
116 measurements, slices with comparable airway and vessel size were selected.  
117 Bronchoconstriction or vasoconstriction was expressed as airway/vessel area as the  
118 percentage of the initial area. A control image was taken before cumulative addition of  
119 U46619 or PGD<sub>2</sub> and frames were recorded every 30 sec for 20 min.

120

### 121 *2.5. Data analysis and statistical procedures*

122 All data are presented as mean  $\pm$  standard error of the mean (S.E.M.). Statistical analyses  
123 were made for paired and unpaired observations by Student's t-test or analyses of variances  
124 (ANOVA) followed by the post hoc tests Bonferroni's t-test. A P-value of less than 0.05 was  
125 considered significant. To provide estimates of maximal effect ( $E_{\max}$ ), midpoint location  
126 ( $pEC_{50}$ ) and Hill slope ( $n_H$ ), agonist concentration-effect curve data from individual tissues  
127 were fitted to the Hill equation using an iterative, least square method (GraphPad Prism, San  
128 Diego, USA). For the Schild plot analysis, the concentration-response curves with antagonists  
129 were set at a global shared maximum assuming competitive antagonism. If the value of the  
130 slope was found not to be significantly different from unity a second fit was performed for the  
131 calculation of  $pK_B$  values with the slope constrained to unity.

132

### 133 *2.6. Drugs and chemical reagents*

134 NaCl, KCl, CaCl<sub>2</sub>, MgSO<sub>4</sub>, NaHCO<sub>3</sub>, KH<sub>2</sub>PO<sub>4</sub> and glucose were obtained from VWR  
135 International (West Chester, Pennsylvania, USA). Histamine dihydrochloride, acetylcholine,  
136 ovalbumin (chicken egg albumin, grade II), agarose, salbutamol, dimethylsulfoxid (DMSO)  
137 were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). 3R-[[4-  
138 fluorophenyl)sulfonyl]amino]-1,2,3,4-tetrahydro-9H-carbazole-9-propanoic acid (BAYu3405,



139 Ramatroban) was purchased from Bayer AG (Wuppertal, Germany). PGD<sub>2</sub>, (4S)-(3-[(3R,S)-  
140 3-cyclohexyl-3-hydroxypropyl]-2,5-dioxo)-4-imidazolidineheptanoic acid (BW245C), LTD<sub>4</sub>,  
141 9,11-dideoxy-9 $\alpha$ ,11 $\alpha$ -methanoepoxy PGF<sub>2 $\alpha$</sub>  (U46619), PGF<sub>2 $\alpha$</sub> , [1S-[1 $\alpha$ ,2 $\alpha$ (Z),3 $\alpha$ ,4 $\alpha$ ]]-7-[3-[[2-  
142 [(phenylamino)carbonyl]hydrazino]methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid  
143 (SQ-29548), 3-[(2-cyclohexyl-2-hydroxyethyl)amino]-2,5-dioxo-1-(phenylmethyl)-4-  
144 imidazolidineheptanoic acid (BWA868C), 9 $\alpha$ ,11 $\beta$ -PGF<sub>2</sub> and 13,14-dihydro-15-keto (DK)-  
145 PGD<sub>2</sub> were bought from Cayman Chemical (Ann Arbor, MI, USA). LTD<sub>4</sub> was from Cascade  
146 Biochemicals Ltd. (Reading, UK). The EIA kits for TXB<sub>2</sub> and PGD<sub>2</sub>-mox were obtained from  
147 Cayman Chemicals (Ann Arbor, Michigan, USA). Stock solutions of 1 mM LTD<sub>4</sub> and  
148 prostanoids were dissolved in 50% ethanol-water and then diluted in 20% ethanol-water.  
149 Ovalbumin was dissolved in 0.9% NaCl. The other drugs were dissolved and diluted in  
150 Tyrode's solution or millipure water. Dilutions of drugs were freshly made from the stocks for  
151 each experiment. The drugs were present in the organ bath fluid during the remaining  
152 experiment. 0.1% DMSO was added as a control and did not influence the baseline or  
153 cumulative contractions to ovalbumin.

154 **3. Results**

155 *3.1. The TP receptor is the main contractile receptor for PGD<sub>2</sub>, TXA<sub>2</sub> and PGF<sub>2α</sub>*

156 PGD<sub>2</sub> induced concentration-dependent contractions in the lung parenchyma (Fig. 1A and B).  
157 The contraction ( $79.8 \pm 8.5\%$ ), obtained at the highest concentration used (100  $\mu$ M), was  
158 according to the non-linear regression analysis not the maximal effect. The inability of PGD<sub>2</sub>  
159 to reach the maximum capacity of the tissue contrasted to U46619 (Fig. 1C and D), which  
160 induced a contraction reaching similar or higher maximum effect as the high concentrations  
161 of histamine, acetylcholine and potassium chloride used as the reference at the end of each  
162 experiment ( $108 \pm 4.6\%$ ) and with a 30-fold greater potency than PGD<sub>2</sub> ( $pEC_{50}$ :  $6.68 \pm 0.24$   
163 and  $5.14 \pm 0.22$ , respectively). However, the concentration-response curves for both PGD<sub>2</sub> and  
164 U44619 were markedly shallow with Hill slopes significantly below 1 ( $0.58 \pm 0.04$  and  $0.53 \pm$   
165  $0.05$ ).

166  
167 When preparations were treated with the competitive TP receptor antagonist SQ-29548, the  
168 concentration-response curve to PGD<sub>2</sub> was shifted to the right (Fig. 1A). Pretreatment with  
169 0.1 or 1  $\mu$ M of SQ-29548 gave rise to significantly different  $pEC_{50}$  values ( $4.7 \pm 0.1$  and  $4.2 \pm$   
170  $0.1$ , respectively) compared to control ( $5.4 \pm 0.3$ ; table 1). At these concentrations of SQ-  
171 29548, the Hill slope was significantly higher than for the control. Further experiments with  
172 the combined TP and DP<sub>2</sub> receptor antagonist BAYu3405 (Fig. 1B) also produced  
173 concentration-dependent rightward shifts of the PGD<sub>2</sub> induced concentration-response curve.  
174 Preparations treated with 0.1 or 1  $\mu$ M BAYu3405 displayed significantly different  $pEC_{50}$   
175 values ( $4.3 \pm 0.1$  and  $4.0 \pm 0.2$ , respectively) compared to controls ( $5.2 \pm 0.2$ ; table 1).

176  
177 In preparations pre-treated with 0.1 or 1  $\mu$ M SQ-29548, U46619 displayed a concentration-  
178 dependent shift of the concentration-response curve and the  $pEC_{50}$  values ( $6.0 \pm 0.2$  and  $5.3 \pm$

179 0.2, respectively) were significantly lower than control preparations ( $6.8 \pm 0.2$ ; Fig. 1C).  
180 When BAYu3405 was used as TP receptor antagonist, the same pattern was shown again  
181 with significantly lower  $pEC_{50}$  values for 0.1 or 1  $\mu$ M BAYu3405 ( $5.4 \pm 0.1$  and  $5.0 \pm 0.1$ ,  
182 respectively), compared to control ( $7.2 \pm 0.4$ ; Fig. 1D). To quantify the antagonistic capacity  
183 for SQ-29548 and BAYu3405, Schild plot analysis was performed, assuming that the agonist  
184 curves reached the similar maximum and ignoring the absence of parallel shifts. Although the  
185 antagonists showed a linear regression statistically not deviating from unity it was a tendency  
186 for lower Schild slopes for both SQ-29548 ( $0.89 \pm 0.08$  for  $PGD_2$ ;  $P = 0.250$ ; and  $0.85 \pm 0.18$   
187 for U46619;  $P = 0.521$ ) and BAYu3405 ( $0.71 \pm 0.25$  for  $PGD_2$ ;  $P = 0.375$ ; and  $0.84 \pm 0.13$  for  
188 U46619;  $P = 0.364$ ). The  $pK_B$  values for the experiments with SQ-2954 rendered a 10-fold  
189 differences between  $PGD_2$  and U46619 ( $7.14 \pm 0.08$  and  $8.17 \pm 0.18$ , respectively) whereas  
190 no significant difference was seen in the experiments with BAYu3405 ( $7.82 \pm 0.15$  and  $7.60 \pm$   
191  $0.16$  for  $PGD_2$  and U46619, respectively).

192

193 To test if the difference between  $PGD_2$  and U46619 could relate to metabolism of  $PGD_2$ , into  
194 a compound activating other receptors, the early  $PGD_2$  metabolite  $9\alpha,11\beta$ - $PGF_2$  was studied.  
195  $9\alpha,11\beta$ - $PGF_2$  yielded a weaker response than both U46619 and  $PGD_2$ , reaching only about 30  
196 % of the maximum contraction (Fig. 2A). The low efficacy of  $9\alpha,11\beta$ - $PGF_2$  did not make it  
197 meaningful to calculate  $pEC_{50}$  values. Since there is structural similarity between  $9\alpha,11\beta$ -  
198  $PGF_2$  and the FP receptor agonist  $PGF_{2\alpha}$  (Komoto et al., 2004),  $PGF_{2\alpha}$  may also be involved  
199 in contractions mediated by  $PGD_2$  and its metabolites. The effect of  $PGF_{2\alpha}$  was almost  
200 identical to the effect of its stereoisomer  $9\alpha,11\beta$ - $PGF_2$  (Fig. 2B). Pretreatment with the TP  
201 receptor antagonist SQ-29548 significantly abolished the contractions induced by  $9\alpha,11\beta$ -  
202  $PGF_2$  ( $P < 0.001$ ) (Fig. 2A) and  $PGF_{2\alpha}$  ( $P < 0.001$ ) (Fig. 2B), indicating that  $PGF_{2\alpha}$  acted as a  
203 TP receptor agonist in the guinea pig peripheral lung preparation.

204

205 *3.2. The DP<sub>1</sub> receptor induce a weak relaxation of peripheral lung tissue*

206 The DP<sub>1</sub> receptor has been described to mediate relaxation of vascular and bronchial SMCs  
207 (Norel, 2007). Therefore, it was investigated if the DP<sub>1</sub> receptor may mediate relaxation of  
208 peripheral lung tissue. Lung strips were treated with cumulative concentrations of the DP<sub>1</sub>  
209 receptor agonist BW245C after pre-contraction with 10 nM of LTD<sub>4</sub>. The pre-contracted  
210 strips relaxed at the highest concentration of BW245C (10 μM) (Fig 3A). Based on these  
211 results, experiments with the DP<sub>1</sub> receptor antagonist BWA868C (0.1 and 1 μM) were  
212 performed with the hypothesis that inhibition of the DP<sub>1</sub> receptor should enhance the PGD<sub>2</sub>  
213 induced contractions. However, the BWA868C treated preparations yielded pEC<sub>50</sub> values for  
214 PGD<sub>2</sub> that were not significant different from controls (Fig. 3B and table 2).

215

216 *3.3. The DP<sub>2</sub> receptor induce neither contraction nor relaxation*

217 Since BAYu3405 is both a TP and DP<sub>2</sub> receptor antagonist, the DP<sub>2</sub> receptor mediated  
218 response in the parenchyma was investigated. Cumulative concentrations of the DP<sub>2</sub> receptor  
219 agonist DK-PGD<sub>2</sub> were added to the lung preparations both before and after pre-contraction  
220 with LTD<sub>4</sub>. DK-PGD<sub>2</sub> up to 10 μM generated neither significant contraction nor relaxation of  
221 the peripheral lung tissue (n=5, data not shown).

222

223 *3.4. The effects of PGD<sub>2</sub> and U46619 in airways and pulmonary vessels in precision-cut lung*  
224 *slices*

225 Since the lung parenchyma preparation consists of both airways and vessels, precision cut  
226 lung slices were examined to study the contractile effect of PGD<sub>2</sub> and U46619 in peripheral  
227 airways and pulmonary arteries and veins. Indeed, PGD<sub>2</sub> induced contractile effects in  
228 airways (pEC<sub>50</sub>: 6.8 ± 0.1) as well as both pulmonary veins (pEC<sub>50</sub>: 7.2 ± 0.2) and arteries

229 (pEC<sub>50</sub>: 6.0 ± 0.2; Fig. 4A). U46619 induced similar strong contractile responses but was  
230 significantly more potent in airways (pEC<sub>50</sub>: 8.9 ± 0.2), veins (pEC<sub>50</sub>: 9.2 ± 0.2) and arteries  
231 (pEC<sub>50</sub>: 8.1 ± 0.2) in the lung parenchymal preparation (Fig. 4B).

232

### 233 *3.5. Role of prostanoids on antigen-induced contractions*

234 Since the contractile effect of both PGD<sub>2</sub> and U46619 was mediated through the TP receptor,  
235 experiments with SQ-29548 were performed to study the significance of this receptor in the  
236 early allergic reaction. Thus, cumulative challenge with ovalbumin on parenchymal strips  
237 from sensitized guinea pigs caused a concentration-dependent contraction that reached about  
238 60-70% of the maximum contraction. Pre-treatment with SQ-29548 partly decreased the  
239 ovalbumin-induced contraction (P < 0.01). Analysis of the bath fluid after challenge with  
240 ovalbumin (1 µg/mL) showed that TXB<sub>2</sub> (1021 ± 325 fmol/g; n=6) was released in 20-fold  
241 higher concentration than PGD<sub>2</sub> (53 ± 6 fmol/g; n=6). Synthesis before ovalbumin stimulation  
242 was 61 ± 51 fmol/g for TXB<sub>2</sub> and not detectable for PGD<sub>2</sub>.

243

244

245

246

247

248

249

250

251

252

253

254 **4. Discussion**

255 It was found that the predominant effect of PGD<sub>2</sub> in the peripheral lung is a contractile effect  
256 which is mediated through activation of TP receptors situated on airways as well as arteries  
257 and veins in the parenchymal lung tissue. A minor relaxant effect was found to be mediated  
258 through the DP<sub>1</sub> receptor and no effect was found to be mediated through the DP<sub>2</sub> in the  
259 present study. When inducing ovalbumin activation of sensitized parenchymal strips, both  
260 TXA<sub>2</sub> and PGD<sub>2</sub> were released. Ovalbumin also induced contractions that were partly  
261 mediated through activation of the TP receptor. Thus, a TP receptor antagonist can be useful  
262 to block the contractile action of TXA<sub>2</sub> and PGD<sub>2</sub> in allergic reactions in the airways (Beasley  
263 et al., 1989).

264

265 PGD<sub>2</sub> and the TP receptor agonist U46619 induced both concentration-response curves with  
266 Hill slopes clearly lower than 1, indicating a complexity in the contractile action. One  
267 possibility that could explain these shallow curves is if the effects were due to action through  
268 more than one receptor. However, since the selective TP (SQ-29548 (Abramovitz et al.,  
269 2000); and the dual TP/DP<sub>2</sub> (BAY u3405 (Sugimoto et al., 2005)) receptor antagonists caused  
270 right-ward shifts of the concentration-response curve to PGD<sub>2</sub>, this indicated, as in line with  
271 previous observations (Hamid-Bloomfield et al., 1990; McKenniff et al., 1991), that the TP  
272 receptor mediates the main part of the PGD<sub>2</sub>-induced contraction. As U46619 induced both  
273 stronger contraction and was more potent than PGD<sub>2</sub>, the data indicated, in accordance with  
274 the much lower affinity for PGD<sub>2</sub> to the TP receptor (Abramovitz et al., 2000), that the  
275 responses were mediated through the TP receptor. On the other hand, the curves for the  
276 antagonist were not shifted in parallel. Although fully concentration-response curves of PGD<sub>2</sub>  
277 were not able to obtain due to the high concentration needed, a clear trend to increased Hill  
278 slope with increasing concentration of antagonists was found for both the agonists, mutually

279 with SQ-29548 and BAY u3405. It is possible that the reason for this increase is due to that  
280 both PGD<sub>2</sub> and U46619 have shown the capacity to bind to almost all prostanoid receptors  
281 (Abramovitz et al., 2000). Thus, at the higher concentrations of the agonists used for the  
282 antagonist experiments, the activation of the TP receptor simultaneously with one or more  
283 prostanoid receptors cause an additional or a synergistic effect.

284

285 Although the Schild plot slopes did not significantly deviate from unity there was a trend to  
286 lower slope values, which may be due to actions of more than one receptor, especially at the  
287 higher concentrations of the agonists. Also the discrepancy of the pK<sub>B</sub> values for SQ-29548  
288 and not BAY u3405 between the agonists can indicate that another receptor than the TP  
289 receptor is activated. The pK<sub>B</sub> values for BAY u3405 (7.82 and 7.60 for PGD<sub>2</sub> and U46619,  
290 respectively) are in line with presented earlier in guinea pig lung strips (7.7 with U46619 as  
291 agonist; (Norman et al., 1992)) whereas the pK<sub>B</sub> values for SQ-29548 was 10 fold lower for  
292 BAY u3405 (7.14) than for U46619 (8.17) as agonists (range from 7.7 to 8.7 in guinea pig;  
293 (Dube et al., 1992; Norman et al., 1992). Since it has been shown that U46619 do not activate  
294 the DP<sub>2</sub> receptor (Monneret et al., 2001), the lower pK<sub>B</sub> value for SQ-29548 from the PGD<sub>2</sub>  
295 experiments can be due to activation of DP<sub>2</sub> receptors. However, as described in this study, a  
296 negligible effect is shown when this receptor is selectively activated. Thus, from these  
297 experiments the reason for the difference of the pK<sub>B</sub> values for SQ-29548 cannot be  
298 completely concluded. Taking all these complexities of the actions of the agonists both in  
299 absence and presence of antagonism in consideration, the clear antagonism with both these  
300 known TP receptor antagonists indicates that the main action of PGD<sub>2</sub> goes through the TP  
301 receptor.

302

303 The major PGD<sub>2</sub> metabolite, 9 $\alpha$ ,11 $\beta$ -PGF<sub>2</sub>, induced a weak contraction of the peripheral lung  
304 that was blocked by SQ-29548 indicating that the breakdown of PGD<sub>2</sub> in this assay do not  
305 cause activation of any further receptor then PGD<sub>2</sub> by itself. PGF<sub>2 $\alpha$</sub>  is a stereoisomer to  
306 9 $\alpha$ ,11 $\beta$ -PGF<sub>2</sub> and closely structurally related to PGD<sub>2</sub> (Sandig et al., 2006). Thus, one  
307 possibility was that PGD<sub>2</sub> also acted through FP receptors (Kiryama et al., 1997). Since there  
308 are no specific receptor antagonists available for the FP receptor we chose to investigate how  
309 the response to PGF<sub>2 $\alpha$</sub>  could be blocked by SQ-29548. However, as the effect of PGF<sub>2 $\alpha$</sub>  was  
310 abolished by SQ-29548 it is unlikely that PGD<sub>2</sub> mediate any major effect through the FP  
311 receptor. Altogether, these data implicate that PGF<sub>2 $\alpha$</sub> , which along with PGD<sub>2</sub> has been shown  
312 to be released after antigen challenge in guinea pig lung (Dawson et al., 1976), also is part of  
313 the TP receptor mediated constriction of the peripheral lung.

314

315 The DP<sub>1</sub> receptor has been shown to mediate relaxation of the bronchioles in response to  
316 PGD<sub>2</sub> and may thus serve as protection in a situation of bronchoconstriction (Norel et al.,  
317 1999). Studies have also shown that rabbit jugular vein preparations treated with the DP<sub>1</sub>  
318 receptor agonist BW245C relaxed concentration-dependently (Lydford et al., 1996).  
319 Nevertheless, the DP<sub>1</sub> receptor agonist only weakly relaxed the parenchymal lung tissue in the  
320 present study. Furthermore, the DP<sub>1</sub> receptor antagonist BWA868C did not affect the PGD<sub>2</sub>  
321 induced contractions. Since the DP<sub>2</sub> receptor agonist did not induce contractions or relaxations  
322 in this lung preparation, this point towards that the main action for PGD<sub>2</sub> on airway  
323 inflammation is the known induction of chemotaxis of eosinophils, basophils and Th2 cells in  
324 guinea pigs (Liu et al., 2005) rather than direct responses on the airway smooth muscle.

325

326 During the control situation, both agonists induced shallow concentration-response curves in  
327 the parenchymal strips. Except for activation on more than one receptor, these shallow curves



328 can be due to the action of several smooth muscle components, such as small airways and  
329 vessels (Evans and Adler, 1981), that not react with similar potencies and maximal effects. In  
330 the precision-cut lung slice experiments, the potency difference between PGD<sub>2</sub> and U44619  
331 of the three studied components was similar as in the parenchymal strips (approximately 100-  
332 fold) indicating similar contractile actions for the agonists during the control situation.  
333 However, a clear difference was obtained between the components, especially the pulmonary  
334 arteries compared to the airways and pulmonary veins with a both lower potency and maximal  
335 effect in the arteries, suggesting that this is the reason for the shallow control curves for both  
336 PGD<sub>2</sub> and U46619. Actually, it is indicated that not only airways and vessels but also pleural  
337 cells, alveolar ducts and interstitial cells are activated by TP receptor agonists (Wong et al.,  
338 1992). Thus, it is possible that these more peripheral located contractile units, which not  
339 easily can be measured in precision-cut lung slices, together with the airways and vessels are  
340 activated in a serial manner causing the described shallow concentration-response curves for  
341 the two agonists.

342

343 Allergen-challenge with ovalbumin showed that both PGD<sub>2</sub> and TXA<sub>2</sub>, the latter measured as  
344 TXB<sub>2</sub>, are released from the distal lung tissue and may be important mediators of the allergen-  
345 induced bronchoconstriction in the peripheral lung. Previous studies in this model have shown  
346 that also histamine, leukotrienes and PGE<sub>2</sub> are generated after ovalbumin stimulation (Larsson  
347 et al., 2009). The 20-fold higher level of TXB<sub>2</sub> in this study is similar to the levels which have  
348 been seen in effluent from ovalbumin exposed isolated perfused and ventilated guinea pig  
349 lung (Selg et al., 2008). That TXA<sub>2</sub> also is released from mast cells has been shown in studies  
350 of the human mast cell line HMC-1 (Macchia et al., 1995). However, human purified sinus  
351 mast cells showed a 10-fold higher level of PGD<sub>2</sub> than TXB<sub>2</sub> after IgE stimulation (Mita et  
352 al., 1999) suggesting that clear species or anatomical differences exist.

353

354 Previously it has been shown that anti-histamine or 5-LO inhibitor alone has no inhibitory  
355 effect on the antigen-induced contraction in guinea pig lung parenchyma (Jonsson and  
356 Dahlen, 1994; Larsson et al., 2007). Furthermore, it has been shown that the allergen induced  
357 contraction in the peripheral lung of the guinea pig needs to be antagonised or inhibited via  
358 several mediator pathways in order to significantly attenuate the contraction (Larsson et al.,  
359 2007; Ressmeyer et al., 2006). However, in the present study we found that the TP receptor  
360 antagonist significantly inhibited part of the ovalbumin-induced contractions. Thus, the  
361 experimental results suggest that TP receptors mediate a significant component of the  
362 allergen-induced contractions in this model of the peripheral lung. The explanation, indicated  
363 by our findings, may be that several COX-products released by the antigen-challenge (PGD<sub>2</sub>,  
364 TXA<sub>2</sub> and PGF<sub>2α</sub>; (Dawson et al., 1976) all act on TP receptors.

365

366 The present study highlights that the parenchymal constriction induced by PGD<sub>2</sub> should be  
367 attributed to its properties as a TP receptor agonist. Even though PGD<sub>2</sub> may have minor role  
368 as an agonist in the allergen-induced contraction in guinea pigs, since it is both released in  
369 lower amount and have lesser effect than TXA<sub>2</sub>, the role in human may be of great importance  
370 due to the high amount of mast cell release (Mita et al., 1999). Accordingly, the contractile  
371 effect of PGD<sub>2</sub> in human airways has also been shown to be antagonized by BAY u3405  
372 (Magnussen et al., 1992), which can be important to bear in mind when considering the  
373 treatment of early asthmatic responses. Furthermore, the results here are consistent with other  
374 studies showing that the allergen response needs to be antagonised or inhibited via several  
375 mechanisms to attenuate the contraction (Jonsson and Dahlen, 1994; Roquet et al., 1997; Selg  
376 et al., 2009). In this concept, therapy with TP receptor antagonists should be considered as  
377 one important component to reduce early asthmatic responses in patients.

378 **Acknowledgement**

379 We would like to express our gratitude to Margareta Andersson for skillful technical  
380 assistance and we would like to thank Swedish Research Council in Medicine and Health,  
381 Swedish Heart and Lung foundation, Vinnova Chronic inflammation - diagnostic and therapy  
382 (CIDaT), the Crafoord Foundation, the Stockholm County Council Research Funds (ALF),  
383 Karolinska Institutet , the Tore Nilsson Foundation and the Swedish Society of Medicine for  
384 financial support.

385

386

387

388

389

390

391

392

393

394

395

396

397

398

399

400

401

402

403 **References**

- 404 Abe, H., Takeshita, T., Nagata, K., Arita, T., Endo, Y., Fujita, T., Takayama, H., Kubo, M.,  
405 Sugamura, K., 1999. Molecular cloning, chromosome mapping and characterization of  
406 the mouse CRTH2 gene, a putative member of the leukocyte chemoattractant receptor  
407 family. *Gene* 227, 71-77.
- 408 Abramovitz, M., Adam, M., Boie, Y., Carriere, M., Denis, D., Godbout, C., Lamontagne, S.,  
409 Rochette, C., Sawyer, N., Tremblay, N.M., Belley, M., Gallant, M., Dufresne, C., Gareau,  
410 Y., Ruel, R., Juteau, H., Labelle, M., Ouimet, N., Metters, K.M., 2000. The utilization of  
411 recombinant prostanoid receptors to determine the affinities and selectivities of  
412 prostaglandins and related analogs. *Biochim. Biophys. Acta* 1483, 285-293.
- 413 Andersson, C.K., Mori, M., Bjermer, L., Lofdahl, C.G., Erjefalt, J.S., 2009. Novel site-  
414 specific mast cell subpopulations in the human lung. *Thorax* 64, 297-305.
- 415 Balzar, S., Fajt, M.L., Comhair, S.A., Erzurum, S.C., Bleecker, E., Busse, W.W., Castro, M.,  
416 Gaston, B., Israel, E., Schwartz, L.B., Curran-Everett, D., Moore, C.G., Wenzel, S.E.,  
417 Mast cell phenotype, location, and activation in severe asthma: data from the severe  
418 asthma research program. *Am. J. Respir. Crit. Care Med.* 183, 299-309.
- 419 Beasley, R.C., Featherstone, R.L., Church, M.K., Rafferty, P., Varley, J.G., Harris, A.,  
420 Robinson, C., Holgate, S.T., 1989. Effect of a thromboxane receptor antagonist on  
421 PGD<sub>2</sub>- and allergen-induced bronchoconstriction. *J. Appl. Physiol.* 66, 1685-1693.
- 422 Canning, B.J., Chou, Y., 2008. Using guinea pigs in studies relevant to asthma and COPD.  
423 *Pulm. Pharmacol. Ther.* 21, 702-720.
- 424 Capra, V., Habib, A., Accomazzo, M.R., Ravasi, S., Citro, S., Levy-Toledano, S., Nicosia, S.,  
425 Rovati, G.E., 2003. Thromboxane prostanoid receptor in human airway smooth muscle  
426 cells: a relevant role in proliferation. *Eur. J. Pharmacol.* 474, 149-159.

427 Coleman, R.A., Smith, W.L., Narumiya, S., 1994. International Union of Pharmacology  
428 classification of prostanoid receptors: properties, distribution, and structure of the  
429 receptors and their subtypes. *Pharmacol. Rev.* 46, 205-229.

430 Dahlen, S.E., Kumlin, M., 2004. Monitoring mast cell activation by prostaglandin D2 in vivo.  
431 *Thorax* 59, 453-455.

432 Dawson, W., Boot, J.R., Cockerill, A.F., Mallen, D.N., Osborne, D.J., 1976. Release of novel  
433 prostaglandins and thromboxanes after immunological challenge of guinea pig lung.  
434 *Nature* 262, 699-702.

435 Dube, G.P., Mais, D.E., Jakubowski, J.A., Brune, K.A., Utterback, B.G., True, T.A.,  
436 Rinkema, L.E., Kurtz, W.L., 1992. In vitro characterization of a novel TXA<sub>2</sub>/PGH<sub>2</sub>  
437 receptor ligand (S-145) in platelets and vascular and airway smooth muscle. *J.*  
438 *Pharmacol. Exp. Ther.* 262, 784-791.

439 Evans, J.N., Adler, K.B., 1981. The lung strip: evaluation of a method to study contractility of  
440 pulmonary parenchyma. *Exp. Lung. Res.* 2, 187-195.

441 Hamid-Bloomfield, S., Payne, A.N., Petrovic, A.A., Whittle, B.J., 1990. The role of  
442 prostanoid TP- and DP-receptors in the bronchoconstrictor effect of inhaled PGD<sub>2</sub> in  
443 anaesthetized guinea-pigs: effect of the DP-antagonist BW A868C. *Br. J. Pharmacol.*  
444 100, 761-766.

445 Held, H.D., Martin, C., Uhlig, S., 1999. Characterization of airway and vascular responses in  
446 murine lungs. *Br. J. Pharmacol.* 126, 1191-1199.

447 Jonsson, E.W., Dahlen, S.E., 1994. Interactions between leukotrienes and histamine in the  
448 anaphylactic contraction of guinea pig lung parenchyma. *J. Pharmacol. Exp. Ther.* 271, 615-  
449 623.

450 Kiriyaama, M., Ushikubi, F., Kobayashi, T., Hirata, M., Sugimoto, Y., Narumiya, S., 1997.  
451 Ligand binding specificities of the eight types and subtypes of the mouse prostanoid  
452 receptors expressed in Chinese hamster ovary cells. *Br. J. Pharmacol.* 122, 217-224.

453 Komoto, J., Yamada, T., Watanabe, K., Takusagawa, F., 2004. Crystal structure of human  
454 prostaglandin F synthase (AKR1C3). *Biochemistry* 43, 2188-2198.

455 Larsson, A.K., Back, M., Hjoberg, J., Dahlen, S.E., 2005. Inhibition of nitric-oxide synthase  
456 enhances antigen-induced contractions and increases release of cysteinyl-leukotrienes in  
457 guinea pig lung parenchyma: nitric oxide as a protective factor. *J. Pharmacol. Exp. Ther.*  
458 315, 458-465.

459 Larsson, A.K., Back, M., Lundberg, J.O., Dahlen, S.E., 2009. Specific mediator inhibition by  
460 the NO donors SNP and NCX 2057 in the peripheral lung: implications for allergen-  
461 induced bronchoconstriction. *Respir. Res.* 10, 46.

462 Larsson, A.K., Fumagalli, F., DiGennaro, A., Andersson, M., Lundberg, J., Edenius, C.,  
463 Govoni, M., Monopoli, A., Sala, A., Dahlen, S.E., Folco, G.C., 2007. A new class of  
464 nitric oxide-releasing derivatives of cetirizine; pharmacological profile in vascular and  
465 airway smooth muscle preparations. *Br. J. Pharmacol.* 151, 35-44.

466 Liu, F., Gonzalo, J.A., Manning, S., O'Connell, L.E., Fedyk, E.R., Burke, K.E., Elder, A.M.,  
467 Pulido, J.C., Cao, W., Tayber, O., Qiu, Y., Ghosh, S., Ocain, T.D., Hodge, M.R., Suzuki-  
468 Yagawa, Y., 2005. Pharmacological characterization of guinea pig chemoattractant  
469 receptor-homologous molecule expressed on Th2 cells (CRTH2). *Prostaglandins Other*  
470 *Lipid Mediat.* 76, 133-147.

471 Liu, M.C., Hubbard, W.C., Proud, D., Stealey, B.A., Galli, S.J., Kagey-Sobotka, A., Bleecker,  
472 E.R., Lichtenstein, L.M., 1991. Immediate and late inflammatory responses to ragweed  
473 antigen challenge of the peripheral airways in allergic asthmatics. Cellular, mediator, and  
474 permeability changes. *Am. Rev. Respir. Dis.* 144, 51-58.

475 Lydford, S.J., McKechnie, K.C., Leff, P., 1996. Interaction of BW A868C, a prostanoid DP-  
476 receptor antagonist, with two receptor subtypes in the rabbit isolated saphenous vein.  
477 Prostaglandins 52, 125-139.

478 Macchia, L., Hamberg, M., Kumlin, M., Butterfield, J.H., Haeggstrom, J.Z., 1995.  
479 Arachidonic acid metabolism in the human mast cell line HMC-1: 5-lipoxygenase gene  
480 expression and biosynthesis of thromboxane. Biochim. Biophys. Acta 1257, 58-74.

481 Magnussen, H., Boerger, S., Templin, K., Baunack, A.R., 1992. Effects of a thromboxane-  
482 receptor antagonist, BAY u 3405, on prostaglandin D2- and exercise-induced  
483 bronchoconstriction. J. Allergy Clin. Immunol. 89, 1119-1126.

484 Matsuoka, T., Hirata, M., Tanaka, H., Takahashi, Y., Murata, T., Kabashima, K., Sugimoto,  
485 Y., Kobayashi, T., Ushikubi, F., Aze, Y., Eguchi, N., Urade, Y., Yoshida, N., Kimura, K.,  
486 Mizoguchi, A., Honda, Y., Nagai, H., Narumiya, S., 2000. Prostaglandin D2 as a  
487 mediator of allergic asthma. Science 287, 2013-2017.

488 McKenniff, M.G., Norman, P., Cuthbert, N.J., Gardiner, P.J., 1991. BAY u3405, a potent and  
489 selective thromboxane A2 receptor antagonist on airway smooth muscle in vitro. Br. J.  
490 Pharmacol. 104, 585-590.

491 Mita, H., Ishii, T., Akiyama, K., 1999. Generation of thromboxane A2 from highly purified  
492 human sinus mast cells after immunological stimulation. Prostaglandins Leukot. Essent.  
493 Fatty Acids 60, 175-180.

494 Monneret, G., Gravel, S., Diamond, M., Rokach, J., Powell, W.S., 2001. Prostaglandin D2 is  
495 a potent chemoattractant for human eosinophils that acts via a novel DP receptor. Blood  
496 98, 1942-1948.

497 Norel, X., 2007. Prostanoid receptors in the human vascular wall. ScientificWorldJournal 7,  
498 1359-1374.

499 Norel, X., Walch, L., Labat, C., Gascard, J.P., Dulmet, E., Brink, C., 1999. Prostanoid  
500 receptors involved in the relaxation of human bronchial preparations. *Br. J. Pharmacol.*  
501 126, 867-872.

502 Norman, P., Cuthbert, N.J., McKenniff, M.G., Gardiner, P.J., 1992. The thromboxane  
503 receptors of rat and guinea-pig lung. *Eur. J. Pharmacol.* 229, 171-178.

504 O'Sullivan, S., Dahlen, B., Dahlen, S.E., Kumlin, M., 1996. Increased urinary excretion of the  
505 prostaglandin D2 metabolite 9 alpha, 11 beta-prostaglandin F2 after aspirin challenge  
506 supports mast cell activation in aspirin-induced airway obstruction. *J. Allergy Clin.*  
507 *Immunol.* 98, 421-432.

508 Okano, M., Fujiwara, T., Sugata, Y., Gotoh, D., Masaoka, Y., Sogo, M., Tanimoto, W.,  
509 Yamamoto, M., Matsumoto, R., Eguchi, N., Kiniwa, M., Isik, A.U., Urade, Y., Nishizaki,  
510 K., 2006. Presence and characterization of prostaglandin D2-related molecules in nasal  
511 mucosa of patients with allergic rhinitis. *Am. J. Rhinol.* 20, 342-348.

512 Parameswaran, K., Radford, K., Fanat, A., Stephen, J., Bonnans, C., Levy, B.D., Janssen, L.J.,  
513 Cox, P.G., 2007. Modulation of human airway smooth muscle migration by lipid  
514 mediators and Th-2 cytokines. *Am. J. Respir. Cell Mol. Biol.* 37, 240-247.

515 Ressmeyer, A.R., Larsson, A.K., Vollmer, E., Dahlen, S.E., Uhlig, S., Martin, C., 2006.  
516 Characterisation of guinea pig precision-cut lung slices: comparison with human tissues.  
517 *Eur. Respir. J.* 28, 603-611.

518 Roquet, A., Dahlen, B., Kumlin, M., Ihre, E., Anstren, G., Binks, S., Dahlen, S.E., 1997.  
519 Combined antagonism of leukotrienes and histamine produces predominant inhibition of  
520 allergen-induced early and late phase airway obstruction in asthmatics. *Am. J. Respir.*  
521 *Crit. Care Med.* 155, 1856-1863.



522 Sandig, H., Andrew, D., Barnes, A.A., Sabroe, I., Pease, J., 2006. 9alpha,11beta-PGF2 and its  
523 stereoisomer PGF2alpha are novel agonists of the chemoattractant receptor, CRTH2.  
524 FEBS Lett. 580, 373-379.

525 Selg, E., Andersson, M., Lastbom, L., Ryrfeldt, A., Dahlen, S.E., 2009. Two different  
526 mechanisms for modulation of bronchoconstriction in guinea-pigs by cyclooxygenase  
527 metabolites. Prostaglandins Other Lipid Mediat. 88, 101-110.

528 Selg, E., Lastbom, L., Ryrfeldt, A., Kumlin, M., Dahlen, S.E., 2008. Effects of selective and  
529 non-selective COX inhibitors on antigen-induced release of prostanoid mediators and  
530 bronchoconstriction in the isolated perfused and ventilated guinea pig lung.  
531 Prostaglandins Leukot. Essent. Fatty Acids 78, 89-97.

532 Sugimoto, H., Shichijo, M., Okano, M., Bacon, K.B., 2005. CRTH2-specific binding  
533 characteristics of [3H]ramatroban and its effects on PGD2-, 15-deoxy-Delta12, 14-PGJ2-  
534 and indomethacin-induced agonist responses. Eur. J. Pharmacol. 524, 30-37.

535 Urade, Y., Eguchi, N., 2002. Lipocalin-type and hematopoietic prostaglandin D synthases as a  
536 novel example of functional convergence. Prostaglandins Other Lipid Mediat. 68-69,  
537 375-382.

538 van den Berge, M., ten Hacken, N.H., Cohen, J., Douma, W.R., Postma, D.S., 2011. Small  
539 airway disease in asthma and COPD: clinical implications. Chest 139, 412-423.

540 Vane, J.R., 1971. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-  
541 like drugs. Nat. New Biol. 231, 232-235.

542 Wong, W.S., Bloomquist, S.L., Bendele, A.M., Fleisch, J.H., 1992. Pharmacological and  
543 histological examinations of regional differences of guinea-pig lung: a role of pleural  
544 surface smooth muscle in lung strip contraction. Br. J. Pharmacol. 105, 620-626.

545

546

547 **Figure legends**

548 **Fig. 1.** Contractions induced by cumulative concentrations of (A and B) PGD<sub>2</sub> and (C and D)  
549 TP receptor agonist U46619 in guinea pig lung parenchymal strips. The experiments were  
550 performed in absence or presence of (A and C) the TP receptor antagonists SQ-29548 or (B  
551 and D) BAY u3405 (n=5-6). Data are presented as mean ± S.E.M..

552

553 **Fig. 2.** Contractions induced by cumulative concentrations of (A) 9 $\alpha$ ,11 $\beta$ - PGF<sub>2</sub> and (B)  
554 PGF<sub>2 $\alpha$</sub>  in guinea pig lung parenchymal strips. The experiments were performed in absence or  
555 presence of 1 $\mu$ M of the TP receptor antagonist SQ-29548 (n=5). Data are presented as mean ±  
556 S.E.M..

557

558 **Fig. 3.** Studies of the DP<sub>1</sub> receptor in guinea pig lung parenchymal strips. (A) Effect of  
559 cumulative concentrations of the DP<sub>1</sub> receptor agonist BW245C after precontraction with  
560 LTD<sub>4</sub> 10 nM and (B) effect of the DP<sub>1</sub> receptor antagonist BWA868C on PGD<sub>2</sub> induced  
561 contractions (n=5). Data are presented as mean ± S.E.M..

562

563 **Fig. 4.** Contractile responses to (A) PGD<sub>2</sub> and (B) the TP receptor agonist U46619 in airway  
564 and, pulmonary artery and vein in guinea pig precision cut lung slices. Data presented as %  
565 change of initial luminal area (n=4). Data are presented as mean ± S.E.M..

566

567 **Fig. 5.** Effect of the TP receptor antagonist SQ-29548 on cumulative doses of ovalbumin  
568 (0.001-10  $\mu$ g/ml) in absence and presence of 1  $\mu$ M SQ-29548 (n=4) in guinea pig lung  
569 parenchymal strips. Data are presented as mean ± S.E.M..

**Table 1:** Effects of the TP receptor antagonists on PGD<sub>2</sub> and U46619 induced contractions

<b>Treatment</b>	<b>N</b>	<b>E<sub>max</sub></b>	<b>pEC<sub>50</sub></b>	<b>Hill slope</b>
PGD <sub>2</sub> (Control)	6	65.2±4.8	5.4± 0.3	0.6 ± 0.0
PGD <sub>2</sub> + SQ-29548 0.01µM	6	78.9±0.7	4.9±0.1	0.6±0.0
PGD <sub>2</sub> + SQ-29548 0.1µM	6	72.5±5.2	4.7±0.1 <sup>a</sup>	0.9±0.1 <sup>a</sup>
PGD <sub>2</sub> + SQ-29548 1µM	6	50.1±7.1	4.2±0.1 <sup>c</sup>	1.7±0.1 <sup>c</sup>
PGD <sub>2</sub> (Control)	5	75.7±3.5	5.2±0.2	0.6±0.0
PGD <sub>2</sub> + BAYu3405 0.1µM	5	64.7±8.2	4.3±0.1 <sup>b</sup>	1.4±0.2 <sup>b</sup>
PGD <sub>2</sub> + BAYu3405 1µM	5	49.2±15.1	4.0±0.2 <sup>c</sup>	1.9±0.1 <sup>c</sup>
U46619 (Control)	6	99.1±0.9	6.8±0.2	0.6±0.1
U46619 + SQ-29548 0.01µM	5	99.1±1.0	6.6±0.2	0.7±0.1
U46619 + SQ-29548 0.1µM	5	100.0±0.0	6.0±0.2 <sup>a</sup>	0.8±0.1
U46619 + SQ-29548 1µM	5	100.0±0.0	5.3±0.2 <sup>c</sup>	1.6±0.3 <sup>c</sup>
U46619 (Control)	6	98.6±1.2	7.2±0.4	0.5±0.1
U46619 + BAYu3405 0.01µM	5	99.8±0.2	6.5±0.2	0.7±0.2
U46619 + BAYu3405 0.1µM	5	100.0±0.0	5.4±0.1 <sup>c</sup>	1.1±0.2
U46619 + BAYu3405 1µM	5	97.9±2.1	5.0±0.1 <sup>c</sup>	1.6±0.2 <sup>c</sup>

Calculations of E<sub>max</sub>, pEC<sub>50</sub> and Hill slope presented as mean ± S.E.M.. Significant differences from the agonists (controls) are indicated as <sup>a</sup>P<0.05, <sup>b</sup>P<0.01 or <sup>c</sup>P<0.001.

**Table 2.** Effects of the DP<sub>1</sub> receptor antagonist on PGD<sub>2</sub> induced contractions

<b>Treatment</b>	<b>N</b>	<b>E<sub>max</sub></b>	<b>pEC<sub>50</sub></b>	<b>Hill slope</b>
PGD <sub>2</sub> (Control)	5	83.8 ± 6.4	4.6 ± 0.3	0.4 ± 0.0
PGD <sub>2</sub> + BWA868C 0.1µM	5	81.5 ± 5.9	4.9 ± 0.1	0.5 ± 0.1
PGD <sub>2</sub> + BWA868C 1µM	5	76.9 ± 7.4	5.0 ± 0.3	0.5 ± 0.1

Calculations for E<sub>max</sub>, pEC<sub>50</sub> and Hill slope are presented as mean ± S.E.M..

Figure 1

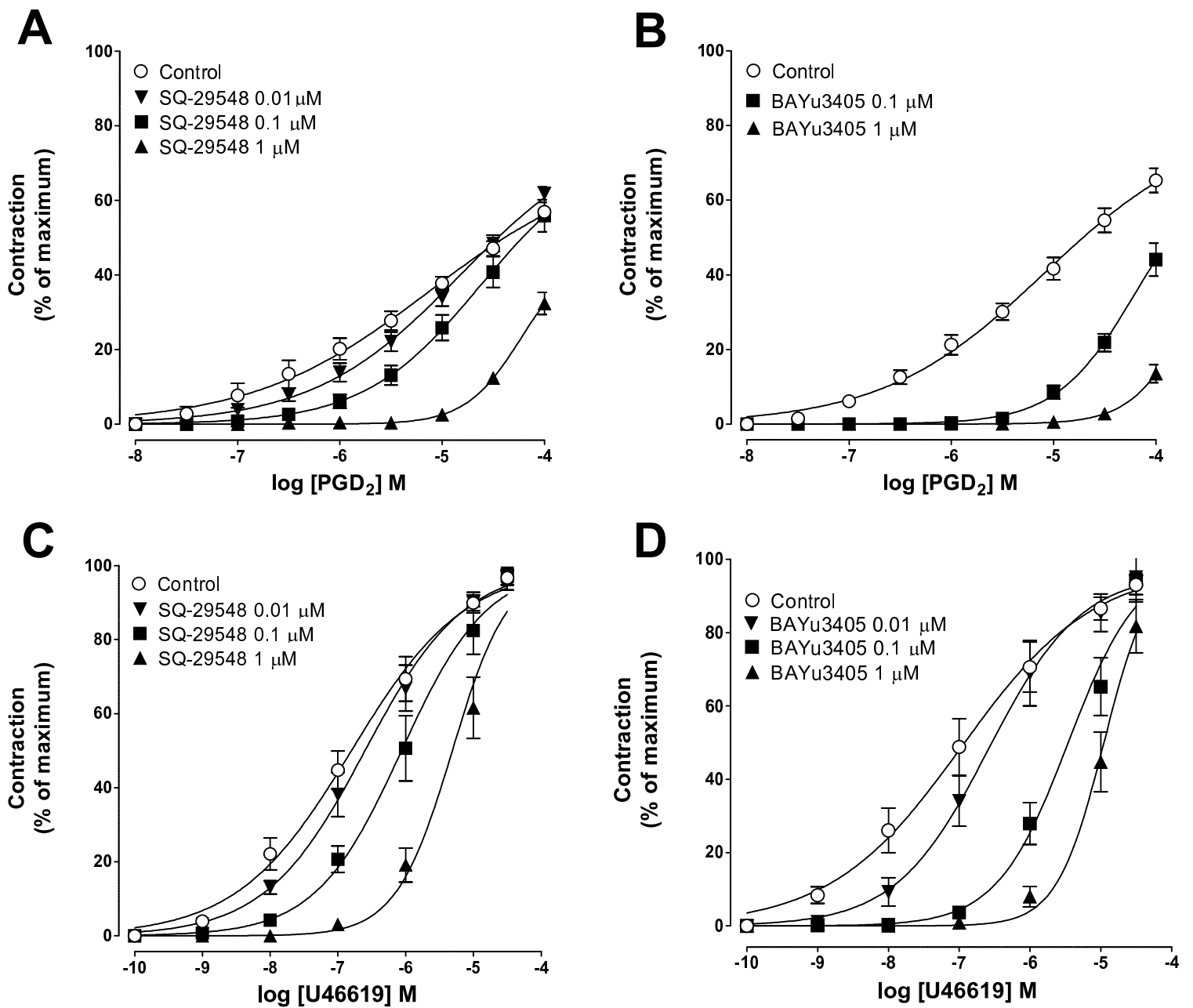


Figure 2

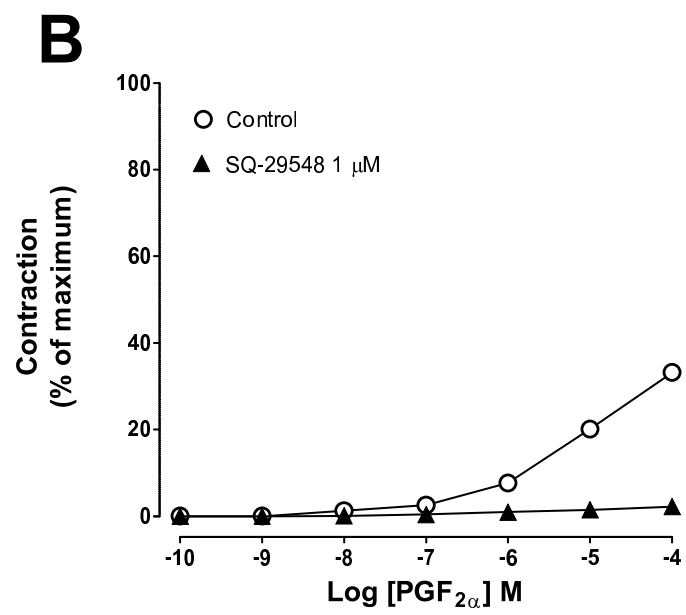
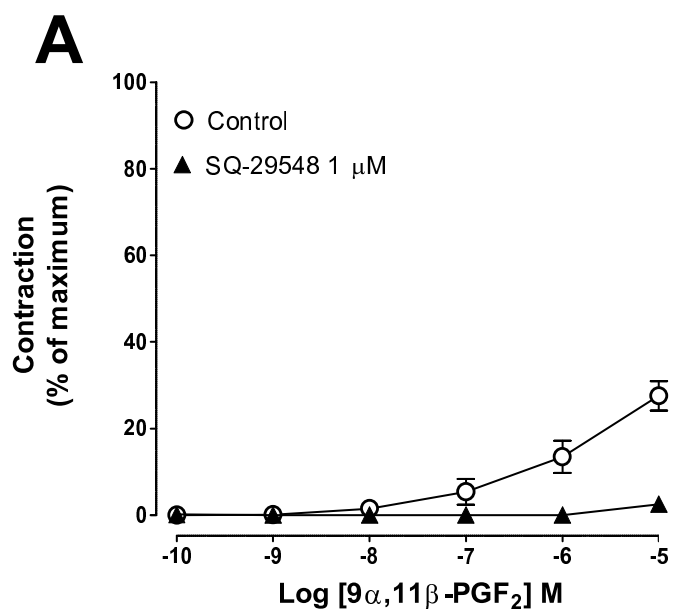


Figure 3

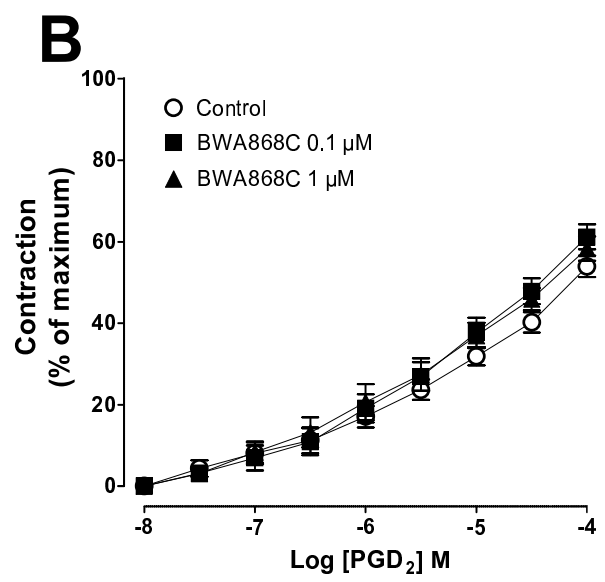
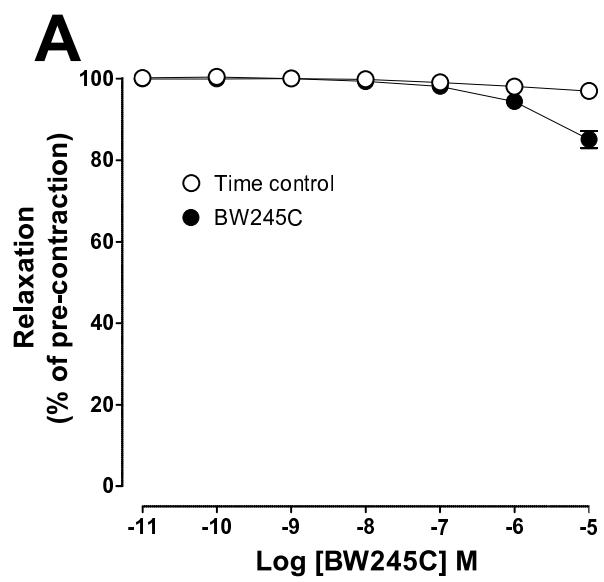


Figure 4

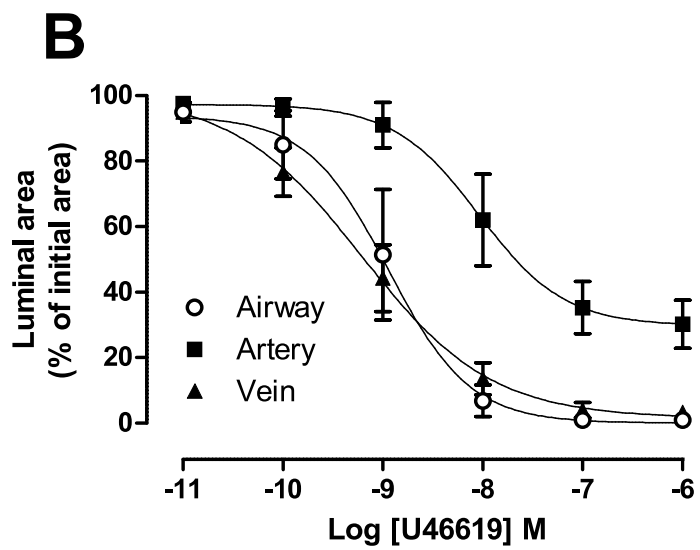
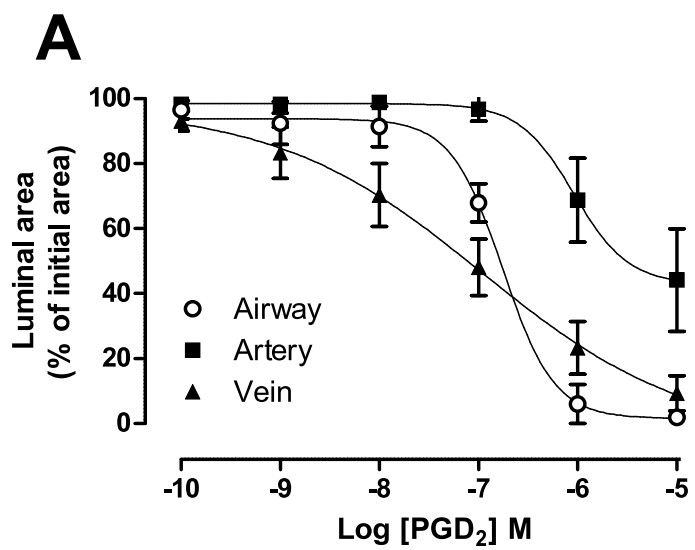




Figure 5

