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Prostaglandin D_2 induces contractions through activation of TP receptors in peripheral lung tissue from the guinea pig

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Abstract

Prostaglandin D_2 (PGD_2), released through mast cell activation, is used as a non-invasive biomarker in patients with asthma. Since PGD_2 can elicit opposing effects on airway tone via activation of the PGD_2 receptors DP_1 and DP_2 as well as the thromboxane receptor TP, the aim of this study was to characterize the receptors that are activated by PGD_2 in the guinea pig lung parenchyma. PGD_2 and the thromboxane analogue U46619 induced concentration-dependent contractions. U46619 was more potent and caused stronger effect than PGD_2. The specific TP receptor antagonist SQ-29548 and the combined TP and DP_2 receptor antagonist BAYu3405 concentration-dependently shifted the curves for both agonists to the right. The DP_1 receptor agonist BW245 induced a weak relaxation at high concentrations, whereas the DP_1 receptor antagonist BWA868C did not affect the PGD_2 induced contractions. The specific DP_2 receptor agonist 13,14-dihydro-15-keto -PGD_2 showed neither contractile nor relaxant effect in the parenchyma. Furthermore, studies in precision-cut lung slices specified that airways as well as pulmonary arteries and veins contracted to both PGD_2 and U46619. When the lung parenchyma from ovalbumin sensitized guinea pigs were exposed to ovalbumin, both thromboxane B_2 and PGD_2 were released. Ovalbumin also induced maximal contractions at similar level as PGD_2 in the parenchyma, which was partly reduced by SQ-29548. These data show that PGD_2 should be recognized as a TP receptor agonist in the peripheral lung inducing contraction on airways, arteries and veins. Therefore, a TP receptor antagonist can be useful in combination treatment of allergic responses in asthma.

Keywords: Guinea pig; lung parenchyma; ovalbumin; precision cut lung slices; prostaglandin D_2; thromboxane

1. Introduction

Prostaglandin D_2 (PGD_2), secreted during mast cell activation (Dahlen and Kumlin, 2004), and its metabolite 9α-11β $PGF_{2\alpha}$ are used as a non-invasive biomarkers in patients with asthma (O'Sullivan et al., 1996). PGD_2 is part of the acute asthmatic airway response; levels of this mediator can be found within minutes in BAL fluid and at 150-fold higher biologically active levels than before the exacerbation (Liu et al., 1991). In addition to the acute reaction, PGD_2 has through recruitment of inflammatory cells been suggested to contribute to the formation of the chronic asthmatic inflammation and subsequent airway remodelling (Balzar et al., 2011).

PGD_2, generated from arachidonic acid, is converted to PG via cyclooxygenase (COX) (Vane, 1971) and PGD synthase (Urade and Eguchi, 2002). There are two distinct types of PGDS; hematopoietic (H-PGDS) and lipocalin-type (L-PGDS). H-PGDS is highly expressed in mast cells, eosinophils, macrophages, and lymphocytes as well as structural cells such as epithelial cells and fibroblasts, whereas L-PGDS is mainly expressed in the central nervous system and heart (Okano et al., 2006). PGD_2 exits the cell via a carrier-mediated process and activates specific G-protein coupled receptors on target cells. PGD_2 is classified to mediate its effect via the DP_1 (Coleman et al., 1994) and the DP_2 ($CRTH_2$) receptors (Abe et al., 1999), but also known to act via the receptor for tromboxane A_2 (TXA_2), the TP receptor (Hamid-Bloomfield et al., 1990). The DP_1 receptor is widely distributed in airway and vascular smooth muscle, blood platelets, airway epithelium and nervous tissue (Coleman et al., 1994; Matsuoka et al., 2000; Norel et al., 1999). PGD_2 has also an important chemotactic role via activation of the DP_2 receptor (Abe et al., 1999), which is mainly expressed on Th2 cells and eosinophils (Abe et al., 1999) but also on human airway smooth muscle (Abe et al., 1999; Parameswaran et al., 2007). The TP receptors are expressed on bronchial and vascular smooth muscle cells, blood

platelets and myofibroblasts (Capra et al., 2003; Coleman et al., 1994) and are known to mediate a strong and long-lasting contraction in these tissues (Held et al., 1999; Ressmeyer et al., 2006). PGD_2 may thus have broad actions since activation of multiple receptors can elicit theoretically opposing effects on airway tone.

Although the lung parenchyma is a complex tissue, the action in the peripheral lung is of importance to study since asthma is suggested to be a disease of the small airways (van den Berge et al., 2011). Especially the action of PGD_2 is of interest since it has been shown that mast cells are located peripherally around small bronchi, vessels and further out to the alveoli (Andersson et al., 2009) and thus may not only affect airways. The aim of this study was therefore to characterize the receptors that are activated by PGD_2 in the peripheral lung and subsequently investigate the significance of this effect in allergen-induced contractions. The guinea pig parenchyma is particularly suitable as it has been shown to respond to many agonists similar to human (Canning and Chou, 2008; Ressmeyer et al., 2006).

2. Methods

2.1. Animals and ovalbumin-sensitization

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

2.2. Lung parenchymal strips and organ bath experiments

The animals were sacrificed by an overdose of inhaled CO_2 and the heart-lung-package was quickly removed and placed in ice-cold Tyrode's solution (prepared each day, containing NaCl 149.2 mM, KCl 2.7 mM, NaHCO3 11.9 mM, glucose 5.5 mM, $CaCl_2$ 1.8 mM, $MgCl_2$ 0.5 mM, NaH2PO4 0.4 mM). The lung parenchyma was cut parallel to the peripheral margins, yielding four to eight strips, each having a size of 2×2×20 mm and a weight of approximately 60 mg. The parenchymal strips were set up at a resting tension of 4.0 mN in 5 ml organ baths filled with Tyrode's solution, bubbled with carbogen gas (6.5% CO_2 in O_2) to keep a pH of 7.4 at 37°C. Changes in smooth muscle tension, contractions and relaxations, were recorded via isometric force-displacement transducers connected to a Grass polygraph. After an equilibration period of 90 min and washes each 15 min, histamine was added as a control of the parenchymal strip reactivity. Preparations displaying contraction responses less than 1.0 mN to 30 µM of histamine were excluded from further experiments. Another wash and equilibration period between histamine and treatment period was performed. All antagonists were given 15 min before the challenges. For study the effect of different agonists, the parenchymal strip was exposed to cumulative concentrations. To study the relaxation the parenchyma was pre-contracted with 10 nM of LTD_4 generating a 50% contraction. To study the allergic early phase reaction, ovalbumin was added as cumulative

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

2.3. Measurements of released mediators with enzyme immunoassays

A 1 mL aliquot of organ bath fluid was collected from each organ bath and immediately frozen at -20°C. The samples were taken at the end of the equilibration period to obtain basal mediator release from the tissue and at the obtained contractile plateau after challenge with ovalbumin 1000 ng/ml. Enzyme immunoassay (EIA) analyses of the prostanoids TXA_2 and PGD_2 were performed according to the manufacturer's instructions. TXA_2 was measured as the stable metabolite TXB_2. PGD_2 was measured as PGD_2-mox. The assay detection limits in the bath fluid levels were 7.8 pg/ml. The EIA specificity for the different mediators to interfere with each other was less than 0.01%, with the exception of the EIA kit for TXB_2 that cross reacted with PGD_2 (0.53%).

2.4. Precision-cut lung slices

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

hours. The slices were then imaged using an analogue (JAI 2040; JAI Pulnix, Alzenau, Germany) or digital camera (IRB640; Visitron Systems, Munich, Germany). For measurements, slices with comparable airway and vessel size were selected. Bronchoconstriction or vasoconstriction was expressed as airway/vessel area as the percentage of the initial area. A control image was taken before cumulative addition of U46619 or PGD_2 and frames were recorded every 30 sec for 20 min.

2.5. Data analysis and statistical procedures

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

2.6. Drugs and chemical reagents

NaCl, KCl, $CaCl_2$, $MgSO_4$, $NaHCO_3$, KH_2PO_4 and glucose were obtained from VWR International (West Chester, Pennsylvania, USA). Histamine dihydrochloride, acetylcholine, ovalbumin (chicken egg albumin, grade II), agarose, salbutamol, dimethylsulfoxid (DMSO) were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). 3R-[[(4-fluorophenyl)sulfonyl]amino]-1,2,3,4-tetrahydro-9H-carbazole-9-propanoic acid (BAYu3405,

Ramatroban) was purchased from Bayer AG (Wuppertal, Germany). PGD_2, (4S)-(3-[(3R,S)-3-cyclohexyl-3-hydroxypropyl]-2,5-dioxo)-4-imidazolidineheptanoic acid (BW245C), LTD_4, 9,11-dideoxy-9α,11α-methanoepoxy $PGF_{2\alpha}$ (U46619), $PGF_{2\alpha}$, [1S-[1α,2α(Z),3α,4α]]-7-[3-[[2-[(phenylamino)carbonyl]hydrazino]methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid (SQ-29548), 3-[(2-cyclohexyl-2-hydroxyethyl)amino]-2,5-dioxo-1-(phenylmethyl)-4-imidazolidineheptanoic acid (BWA868C), 9α,11β-PGF_2 and 13,14-dihydro-15-keto (DK)-PGD_2 were bought from Cayman Chemical (Ann Arbor, MI, USA). LTD_4 was from Cascade Biochemicals Ltd. (Reading, UK). The EIA kits for TXB_2 and PGD_2-mox were obtained from Cayman Chemicals (Ann Arbor, Michigan, USA). Stock solutions of 1 mM LTD_4 and prostanoids were dissolved in 50% ethanol-water and then diluted in 20% ethanol-water. Ovalbumin was dissolved in 0.9% NaCl. The other drugs were dissolved and diluted in Tyrode's solution or millipure water. Dilutions of drugs were freshly made from the stocks for each experiment. The drugs were present in the organ bath fluid during the remaining experiment. 0.1% DMSO was added as a control and did not influence the baseline or cumulative contractions to ovalbumin.

3. Results

3.1. The TP receptor is the main contractile receptor for PGD_2, TXA_2 and $PGF_{2\alpha}$

PGD_2 induced concentration-dependent contractions in the lung parenchyma (Fig. 1A and B). The contraction (79.8 ± 8.5%), obtained at the highest concentration used (100 µM), was according to the non-linear regression analysis not the maximal effect. The inability of PGD_2 to reach the maximum capacity of the tissue contrasted to U46619 (Fig. 1C and D), which induced a contraction reaching similar or higher maximum effect as the high concentrations of histamine, acetylcholine and potassium chloride used as the reference at the end of each experiment (108 ± 4.6%) and with a 30-fold greater potency than PGD_2 (pEC_{50}: 6.68 ± 0.24 and 5.14 ± 0.22, repectively). However, the concentration-response curves for both PGD_2 and U44619 were markedly shallow with Hill slopes significantly below 1 (0.58 ± 0.04 and 0.53 ± 0.05) .

When preparations were treated with the competitive TP receptor antagonist SQ-29548, the concentration-response curve to PGD_2 was shifted to the right (Fig. 1A). Pretreatment with 0.1 or 1 µM of SQ-29548 gave rise to significantly different pEC_{50} values (4.7 ± 0.1 and 4.2 ± 0.1, respectively) compared to control (5.4 ± 0.3; table 1). At these concentrations of SQ-29548, the Hill slope was significantly higher than for the control. Further experiments with the combined TP and DP_2 receptor antagonist BAYu3405 (Fig. 1B) also produced concentration-dependent rightward shifts of the PGD_2 induced concentration-response curve. Preparations treated with 0.1 or 1 µM BAYu3405 displayed significantly different pEC_{50} values (4.3 ± 0.1 and 4.0 ± 0.2, respectively) compared to controls (5.2 ± 0.2; table 1).

In preparations pre-treated with 0.1 or 1 µM SQ-29548, U46619 displayed a concentration-dependent shift of the concentration-response curve and the pEC_{50} values (6.0 ± 0.2 and 5.3 ±

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

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

3.2. The DP_1 receptor induce a weak relaxation of peripheral lung tissue

The DP_1 receptor has been described to mediate relaxation of vascular and bronchial SMCs (Norel, 2007). Therefore, it was investigated if the DP_1 receptor may mediate relaxation of peripheral lung tissue. Lung strips were treated with cumulative concentrations of the DP_1 receptor agonist BW245C after pre-contraction with 10 nM of LTD_4. The pre-contracted strips relaxed at the highest concentration of BW245C (10 µM) (Fig 3A). Based on these results, experiments with the DP_1 receptor antagonist BWA868C (0.1 and 1 µM) were performed with the hypothesis that inhibition of the DP_1 receptor should enhance the PGD_2 induced contractions. However, the BWA868C treated preparations yielded pEC_{50} values for PGD_2 that were not significant different from controls (Fig. 3B and table 2).

3.3. The DP_2 receptor induce neither contraction nor relaxation

Since BAYu3405 is both a TP and DP_2 receptor antagonist, the DP_2 receptor mediated response in the parenchyma was investigated. Cumulative concentrations of the DP_2 receptor agonist DK-PGD_2 were added to the lung preparations both before and after pre-contraction with LTD_4. DK-PGD_2 up to 10 µM generated neither significant contraction nor relaxation of the peripheral lung tissue (n=5, data not shown).

3.4. The effects of PGD_2 and U46619 in airways and pulmonary vessels in precision-cut lung slices

Since the lung parenchyma preparation consists of both airways and vessels, precision cut lung slices were examined to study the contractile effect of PGD_2 and U46619 in peripheral airways and pulmonary arteries and veins. Indeed, PGD_2 induced contractile effects in airways (pEC_{50}: 6.8 ± 0.1) as well as both pulmonary veins (pEC_{50}: 7.2 ± 0.2) and arteries

(pEC_{50}: 6.0 ± 0.2; Fig. 4A). U46619 induced similar strong contractile responses but was significantly more potent in airways (pEC50: 8.9 ± 0.2), veins (pEC_{50}: 9.2 ± 0.2) and arteries (pEC_{50}: 8.1± 0.2) in the lung parenchymal preparation (Fig. 4B).

3.5. Role of prostanoids on antigen-induced contractions

Since the contractile effect of both PGD_2 and U46619 was mediated through the TP receptor, experiments with SQ-29548 were performed to study the significance of this receptor in the early allergic reaction. Thus, cumulative challenge with ovalbumin on parenchymal strips from sensitized guinea pigs caused a concentration-dependent contraction that reached about 60-70% of the maximum contraction. Pre-treatment with SQ-29548 partly decreased the ovalbumin-induced contraction ($P < 0.01$). Analysis of the bath fluid after challenge with ovalbumin (1 µg/mL) showed that TXB_2 (1021 ± 325 fmol/g; n=6) was released in 20-fold higher concentration than PGD_2 (53 ± 6 fmol/g; n=6). Synthesis before ovalbumin stimulation was 61 ± 51 fmol/g for TXB_2 and not detectable for PGD_2.

4. Discussion

It was found that the predominant effect of PGD_2 in the peripheral lung is a contractile effect which is mediated through activation of TP receptors situated on airways as well as arteries and veins in the parenchymal lung tissue. A minor relaxant effect was found to be mediated through the DP_1 receptor and no effect was found to be mediated through the DP_2 in the present study. When inducing ovalbumin activation of sensitized parenchymal strips, both TXA_2 and PGD_2 were released. Ovalbumin also induced contractions that were partly mediated through activation of the TP receptor. Thus, a TP receptor antagonist can be useful to block the contractile action of TXA_2 and PGD_2 in allergic reactions in the airways (Beasley et al., 1989).

PGD_2 and the TP receptor agonist U46619 induced both concentration-response curves with Hill slopes clearly lower than 1, indicating a complexity in the contractile action. One possibility that could explain these shallow curves is if the effects were due to action through more than one receptor. However, since the selective TP (SQ-29548 (Abramovitz et al., 2000); and the dual TP/DP_2 (BAY u3405 (Sugimoto et al., 2005)) receptor antagonists caused right-ward shifts of the concentration-response curve to PGD_2, this indicated, as in line with previous observations (Hamid-Bloomfield et al., 1990; McKenniff et al., 1991), that the TP receptor mediates the main part of the PGD_2-induced contraction. As U46619 induced both stronger contraction and was more potent than PGD_2, the data indicated, in accordance with the much lower affinity for PGD_2 to the TP receptor (Abramovitz et al., 2000), that the responses were mediated through the TP receptor. On the other hand, the curves for the antagonist were not shifted in parallel. Although fully concentration-response curves of PGD_2 were not able to obtain due to the high concentration needed, a clear trend to increased Hill slope with increasing concentration of antagonists was found for both the agonists, mutually

with SQ-29548 and BAY u3405. It is possible that the reason for this increase is due to that both PGD_2 and U46619 have shown the capacity to bind to almost all prostanoid receptors (Abramovitz et al., 2000). Thus, at the higher concentrations of the agonists used for the antagonist experiments, the activation of the TP receptor simultaneously with one or more prostanoid receptors cause an additional or a synergistic effect.

Although the Schild plot slopes did not significantly deviate from unity there was a trend to lower slope values, which may be due to actions of more than one receptor, especially at the higher concentrations of the agonists. Also the discrepancy of the pK_B values for SQ-29548 and not BAY u3405 between the agonists can indicate that another receptor than the TP receptor is activated. The pK_B values for BAY u3405 (7.82 and 7.60 for PGD_2 and U46619, respectively) are in line with presented earlier in guinea pig lung strips (7.7 with U46619 as agonist; (Norman et al., 1992)) whereas the pK_B values for SQ-29548 was 10 fold lower for BAY u3405 (7.14) than for U46619 (8.17) as agonists (range from 7.7 to 8.7 in guinea pig; (Dube et al., 1992; Norman et al., 1992). Since it has been shown that U46619 do not activate the DP_2 receptor (Monneret et al., 2001), the lower pK_B value for SQ-29548 from the PGD_2 experiments can be due to activation of DP_2 receptors. However, as described in this study, a negligible effect is shown when this receptor is selectively activated. Thus, from these experiments the reason for the difference of the pK_B values for SQ-29548 cannot be completely concluded. Taking all these complexities of the actions of the agonists both in absence and presence of antagonism in consideration, the clear antagonism with both these known TP receptor antagonists indicates that the main action of PGD_2 goes through the TP receptor.

The major PGD_2 metabolite, 9α,11β-PGF_2, induced a weak contraction of the peripheral lung that was blocked by SQ-29548 indicating that the breakdown of PGD_2 in this assay do not cause activation of any further receptor then PGD_2 by itself. $PGF_{2\alpha}$ is a stereoisomer to 9α,11β-PGF_2 and closely structurally related to PGD_2 (Sandig et al., 2006). Thus, one possibility was that PGD_2 also acted through FP receptors (Kiriyama et al., 1997). Since there are no specific receptor antagonists available for the FP receptor we chose to investigate how the response to $PGF_{2\alpha}$ could be blocked by SQ-29548. However, as the effect of $PGF_{2\alpha}$ was abolished by SQ-29548 it is unlikely that PGD_2 mediate any major effect through the FP receptor. Altogether, these data implicate that $PGF_{2\alpha}$, which along with PGD_2 has been shown to be released after antigen challenge in guinea pig lung (Dawson et al., 1976), also is part of the TP receptor mediated constriction of the peripheral lung.

The DP_1 receptor has been shown to mediate relaxation of the bronchioles in response to PGD_2 and may thus serve as protection in a situation of bronchoconstriction (Norel et al., 1999). Studies have also shown that rabbit jugular vein preparations treated with the DP_1 receptor agonist BW245C relaxed concentration-dependently (Lydford et al., 1996). Nevertheless, the DP_1 receptor agonist only weakly relaxed the parenchymal lung tissue in the present study. Furthermore, the DP_1 receptor antagonist BWA868C did not affect the PGD_2 induced contractions. Since the DP_2 receptor agonist did not induce contractions or relaxations in this lung preparation, this point towards that the main action for PGD_2 on airway inflammation is the known induction of chemotaxis of eosinophils, basophils and Th2 cells in guinea pigs (Liu et al., 2005) rather than direct responses on the airway smooth muscle.

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

can be due to the action of several smooth muscle components, such as small airways and vessels (Evans and Adler, 1981), that not react with similar potencies and maximal effects. In the precision-cut lung slice experiments, the potency difference between PGD_2 and U44619 of the three studied components was similar as in the parenchymal strips (approximately 100-fold) indicating similar contractile actions for the agonists during the control situation. However, a clear difference was obtained between the components, especially the pulmonary arteries compared to the airways and pulmonary veins with a both lower potency and maximal effect in the arteries, suggesting that this is the reason for the shallow control curves for both PGD_2 and U46619. Actually, it is indicated that not only airways and vessels but also pleural cells, alveolar ducts and interstitial cells are activated by TP receptor agonists (Wong et al., 1992). Thus, it is possible that these more peripheral located contractile units, which not easily can be measured in precision-cut lung slices, together with the airways and vessels are activated in a serial manner causing the described shallow concentration-response curves for the two agonists.

Allergen-challenge with ovalbumin showed that both PGD_2 and TXA_2, the latter measured as TXB_2, are released from the distal lung tissue and may be important mediators of the allergen-induced bronchoconstriction in the peripheral lung. Previous studies in this model have shown that also histamine, leukotrienes and PGE_2 are generated after ovalbumin stimulation (Larsson et al., 2009). The 20-fold higher level of TXB_2 in this study is similar to the levels which have been seen in effluent from ovalbumin exposed isolated perfused and ventilated guinea pig lung (Selg et al., 2008). That TXA_2 also is released from mast cells has been shown in studies of the human mast cell line HMC-1 (Macchia et al., 1995). However, human purified sinus mast cells showed a 10-fold higher level of PGD_2 than TXB_2 after IgE stimulation (Mita et al., 1999) suggesting that clear species or anatomical differences exist.

Previously it has been shown that anti-histamine or 5-LO inhibitor alone has no inhibitory effect on the antigen-induced contraction in guinea pig lung parenchyma (Jonsson and Dahlen, 1994; Larsson et al., 2007). Furthermore, it has been shown that the allergen induced contraction in the peripheral lung of the guinea pig needs to be antagonised or inhibited via several mediator pathways in order to significantly attenuate the contraction (Larsson et al., 2007; Ressmeyer et al., 2006). However, in the present study we found that the TP receptor antagonist significantly inhibited part of the ovalbumin-induced contractions. Thus, the experimental results suggest that TP receptors mediate a significant component of the allergen-induced contractions in this model of the peripheral lung. The explanation, indicated by our findings, may be that several COX-products released by the antigen-challenge (PGD_2, TXA_2 and $PGF_{2\alpha}$; (Dawson et al., 1976) all act on TP receptors.

The present study highlights that the parenchymal constriction induced by PGD_2 should be attributed to its properties as a TP receptor agonist. Even though PGD_2 may have minor role as an agonist in the allergen-induced contraction in guinea pigs, since it is both released in lower amount and have lesser effect than TXA_2, the role in human may be of great importance due to the high amount of mast cell release (Mita et al., 1999). Accordingly, the contractile effect of PGD_2 in human airways has also been shown to be antagonized by BAY u3405 (Magnussen et al., 1992), which can be important to bear in mind when considering the treatment of early asthmatic responses. Furthermore, the results here are consistent with other studies showing that the allergen response needs to be antagonised or inhibited via several mechanisms to attenuate the contraction (Jonsson and Dahlen, 1994; Roquet et al., 1997; Selg et al., 2009). In this concept, therapy with TP receptor antagonists should be considered as one important component to reduce early asthmatic responses in patients.

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Figure legends

Fig. 1. Contractions induced by cumulative concentrations of (A and B) PGD_2 and (C and D) TP receptor agonist U46619 in guinea pig lung parenchymal strips. The experiments were performed in absence or presence of (A and C) the TP receptor antagonists SQ-29548 or (B and D) BAY u3405 (n=5-6). Data are presented as mean ± S.E.M..

Fig. 2. Contractions induced by cumulative concentrations of (A) 9α,11β- PGF_2 and (B) $PGF_{2\alpha}$ in guinea pig lung parenchymal strips. The experiments were performed in absence or presence of 1μM of the TP receptor antagonist SQ-29548 (n=5). Data are presented as mean ± S.E.M..

Fig. 3. Studies of the DP_1 receptor in guinea pig lung parenchymal strips. (A) Effect of cumulative concentrations of the DP_1 receptor agonist BW245C after precontraction with LTD_4 10 nM and (B) effect of the DP_1 receptor antagonist BWA868C on PGD_2 induced contractions (n=5). Data are presented as mean ± S.E.M..

Fig. 4. Contractile responses to (A) PGD_2 and (B) the TP receptor agonist U46619 in airway and, pulmonary artery and vein in guinea pig precision cut lung slices. Data presented as % change of initial luminal area (n=4). Data are presented as mean ± S.E.M..

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

Table 1: Effects of the TP receptor antagonists on PGD_2 and U46619 induced contractions

Treatment	N	Emax	pEC50	Hill slope
PGD_2 (Control)	6	65.2±4.8	5.4± 0.3	0.6 ± 0.0
PGD_2 + SQ-29548 0.01µM	6	78.9±0.7	4.9±0.1	0.6±0.0
PGD_2 + SQ-29548 0.1µM	6	72.5±5.2	4.7±0.1[a]	0.9±0.1[a]
PGD_2 + SQ-29548 1µM	6	50.1±7.1	4.2±0.1[c]	1.7±0.1[c]
PGD_2 (Control)	5	75.7±3.5	5.2±0.2	0.6±0.0
PGD_2 + BAYu3405 0.1µM	5	64.7±8.2	4.3±0.1[b]	1.4±0.2[b]
PGD_2 + BAYu3405 1µM	5	49.2±15.1	4.0±0.2[c]	1.9±0.1[c]
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[a]	0.8±0.1
U46619 + SQ-29548 1µM	5	100.0±0.0	5.3±0.2[c]	1.6±0.3[c]
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[c]	1.1±0.2
U46619 + BAYu3405 1µM	5	97.9±2.1	5.0±0.1[c]	1.6±0.2[c]

Calculations of Emax, pEC50 and Hill slope presented as mean ± S.E.M.. Significant differences from the agonists (controls) are indicated as [a] $P<0.05$, [b] $P<0.01$ or [c] $P<0.001$.

Table 2. Effects of the DP_1 receptor antagonist on PGD_2 induced contractions

Treatment	N	Emax	pEC50	Hill slope
PGD_2 (Control)	5	83.8 ± 6.4	4.6 ± 0.3	0.4 ± 0.0
PGD_2 + BWA868C 0.1µM	5	81.5 ± 5.9	4.9 ± 0.1	0.5 ± 0.1
PGD_2 + BWA868C 1µM	5	76.9 ± 7.4	5.0 ± 0.3	0.5 ± 0.1

Calculations for Emax, pEC_{50} and Hill slope are presented as mean ± S.E.M..

Figure 1

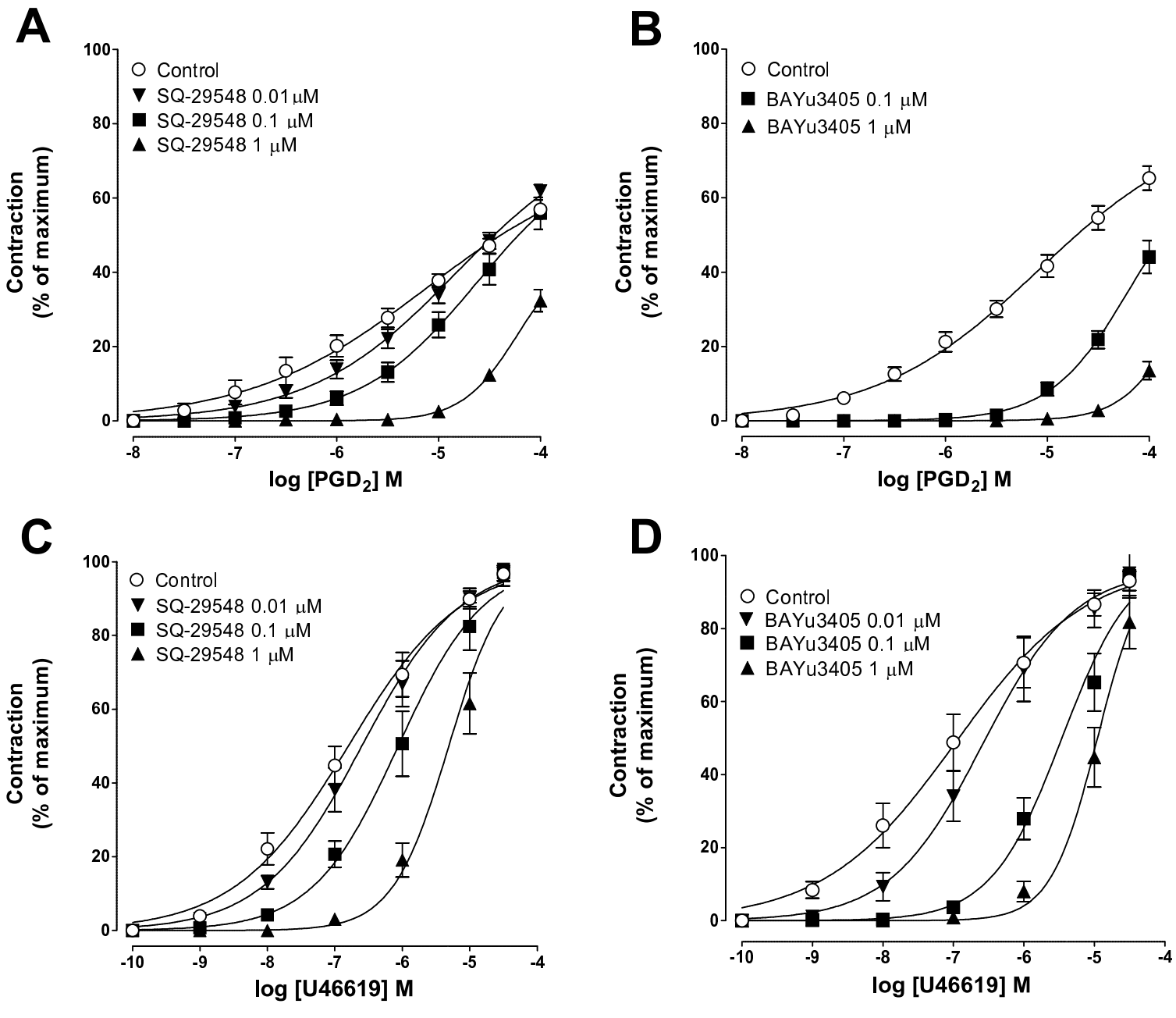
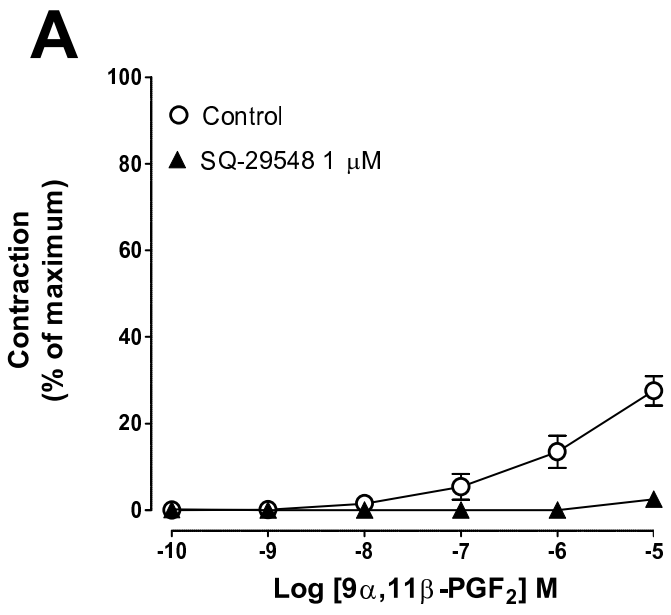

A
Control
SQ-29548 0.01 μM
SQ-29548 0.1 μM
SQ-29548 1 μM
Contraction (% of maximum)
log [PGD2] M
B
Control
BAYu3405 0.1 μM
BAYu3405 1 μM
Contraction (% of maximum)
log [PGD2] M
C
Control
SQ-29548 0.01 μM
SQ-29548 0.1 μM
SQ-29548 1 μM
Contraction (% of maximum)
log [U46619] M
D
Control
BAYu3405 0.01 μM
BAYu3405 0.1 μM
BAYu3405 1 μM
Contraction (% of maximum)
log [U46619] M

Figure 2


A
100
80
60
40
20
0
Control
SQ-29548 1 μM
Contraction
(% of maximum)
-10
-9
-8
-7
-6
-5
Log [9α,11β-PGF2] M

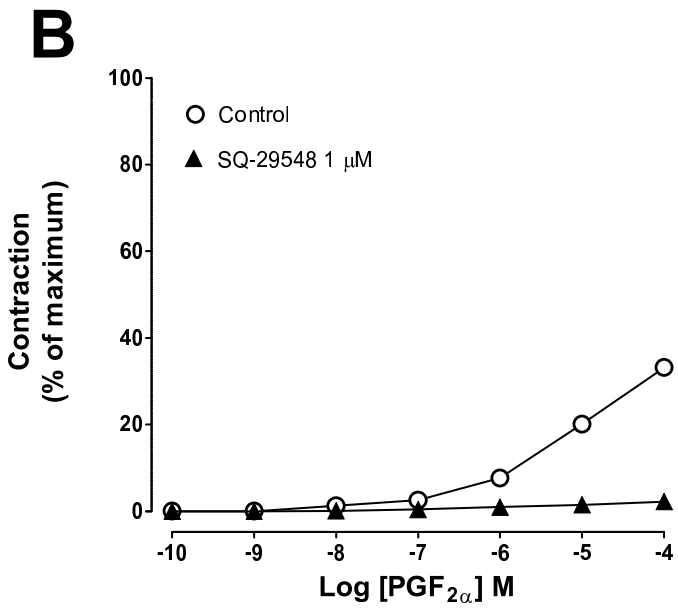

B
100
80
60
40
20
0
Control
SQ-29548 1 μM
Contraction
(% of maximum)
-10
-9
-8
-7
-6
-5
-4
Log [PGF2α] M

Figure 3

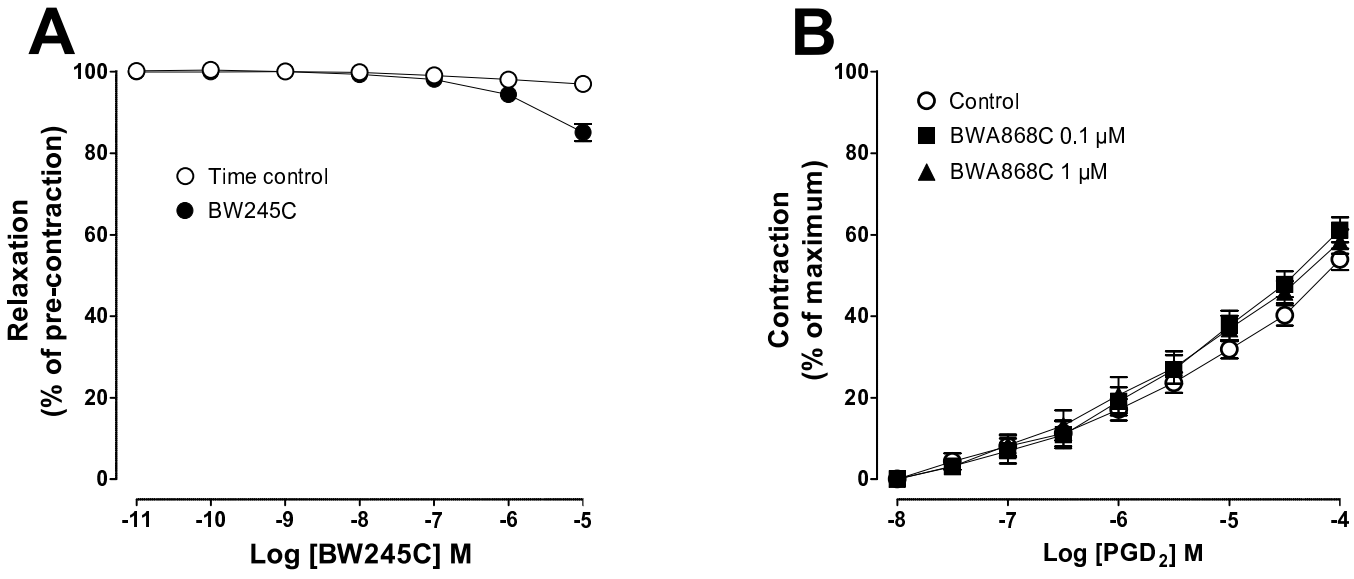

A
Relaxation
(% of pre-contraction)
100
80
60
40
20
0
Time control
BW245C
-11
-10
-9
-8
-7
-6
-5
Log [BW245C] M
B
Contraction
(% of maximum)
100
80
60
40
20
0
Control
BWA868C 0.1 µM
BWA868C 1 µM
-8
-7
-6
-5
-4
Log [PGD2] M

Figure 4

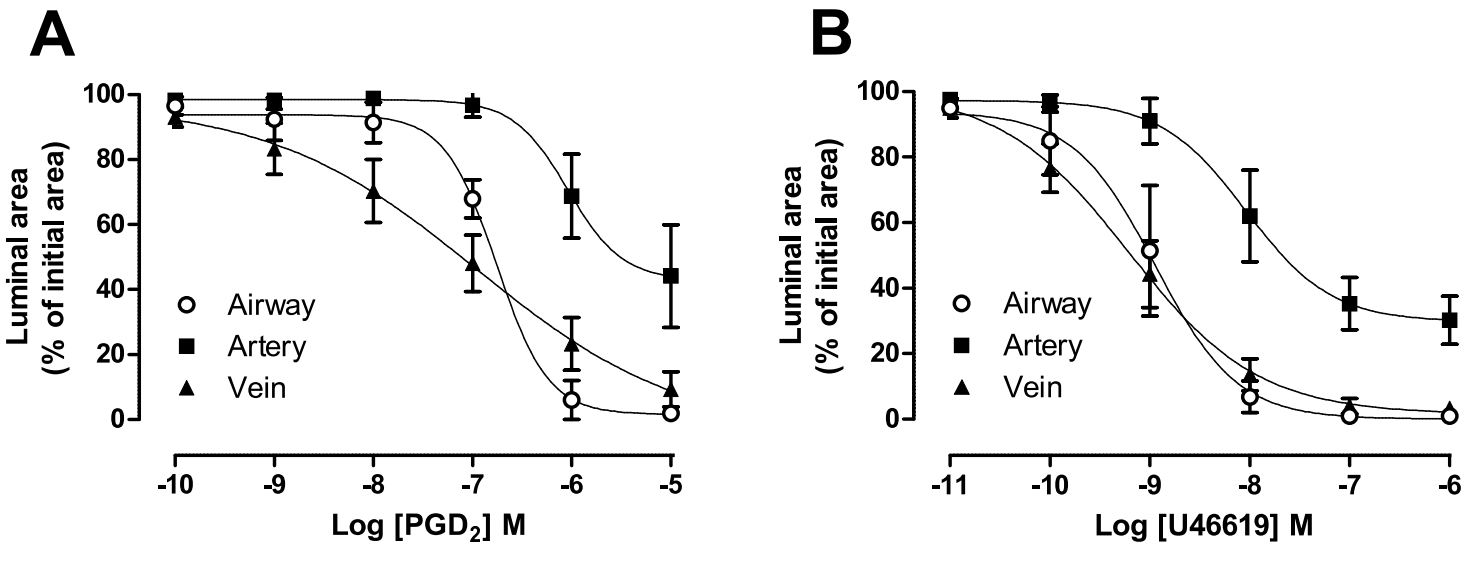
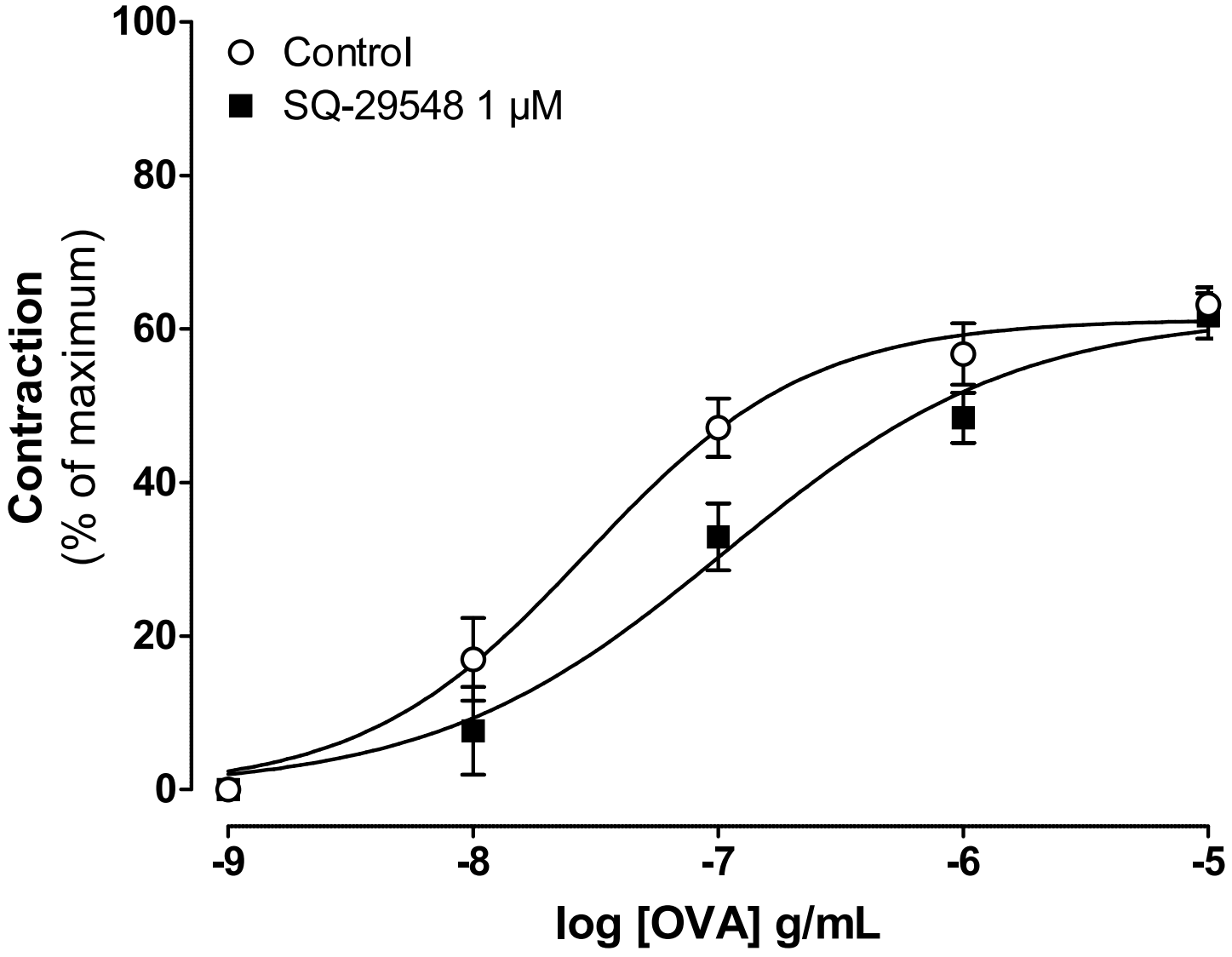

A
Luminal area
(% of initial area)
100
80
60
40
20
0
Airway
Artery
Vein
-10
-9
-8
-7
-6
-5
Log [PGD2] M
B
Luminal area
(% of initial area)
100
80
60
40
20
0
Airway
Artery
Vein
-11
-10
-9
-8
-7
-6
Log [U46619] M

Figure 5


Control
SQ-29548 1 µM
Contraction
(% of maximum)
100
80
60
40
20
0
-9
-8
-7
-6
-5
log [OVA] g/mL