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Characterization of guinea pig precision-cut lung slices: Comparison with human tissues

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Abstract

Precision-cut lung slices (PCLS) allow comparison of airway responses of different species under identical experimental conditions. Our aim was to establish and characterize PCLS from guinea pigs (GP) and to compare them to human PCLS.

Guinea pig PCLS were prepared according to previously published procedures with the exception that the agarose solution and the initial incubation medium contained isoproterenol to avoid *post mortem* airway contraction.

The median effective concentrations (EC_{50} [nM]) for agonist-induced bronchoconstriction in GP and human PCLS were: leukotriene D_4 (1.8, 5.0), thromboxane (16, 1.3), serotonin (69, unresponsive), histamine (217, 2170), and methacholine (231, 234). Allergen-induced bronchoconstriction of passively sensitized PCLS was attenuated by histamine or thromboxane-prostanoid (TP)-receptor antagonists and was almost completely prevented by their combination with leukotriene receptor antagonists. Airways precontracted with methacholine were relaxed by the β-agonist salbutamol or the phosphodiesterase (PDE)-inhibitor IBMX. Studies of both airways and vessels are applicable with EC_{50} values for endothelin-1 of 37 nM (pulmonary arteries), 10 nM (pulmonary veins), and 9.6 nM (airway).

When compared with previous findings in rat and mouse, our data show that GP lungs are a more appropriate model for human airway pharmacology than are lungs from rats or mice.

Keywords: lung explants, airway smooth muscle, 2-photon microscopy, live dead staining, ovalbumin, eicosanoids

Introduction

The airway pharmacology is strikingly dissimilar among species. For example, rodent airways do not or only weakly respond to leukotrienes (1), a mediator that readily causes bronchoconstriction in humans (2) and guinea pigs (3;4). Guinea pigs, which are no rodents (5), are widely used in pulmonary pharmacology, because their airways' responsiveness to mediators and drugs is thought to resemble human airways more closely than do those of mice or rats (6;7). However, most of the previous studies have focussed either on *in vivo* experiments or on large airway preparations, both of which models are difficult to translate to studies with humans, making direct interspecies comparisons difficult. In addition, the significance of peripheral airways for the pathology of asthma has only recently been appreciated (8-11).

A recently developed alternative to the classical pharmacological models is the precision-cut lung slice (PCLS) model. Originally devised for toxicological studies (12-14), we have established the utilization of PCLS from rats, mice and humans for studies of peripheral airway pharmacology (1;9;15). The PCLS model has many advantages: (i) Since slices are viewed under the microscope, it is possible to study airways and vessels of different size and anatomical location. For instance, rat and human PCLS small airways respond stronger than larger airways to methacholine (Mch) (15), serotonin (8), thromboxane (16) and allergen (8;9), but not to endothelin-1 (17). (ii) The slices are usually studied in 24-well plates requiring only 500-1000 µl bathing solution, which permits to examine expensive or rare drugs. From one lung up to 30 slices can be prepared which does not only limit the use of experimental animals, but can also help to reduce experimental error as it allows statistical blocking. (iii) The slices are available for experimentation for at least 3 days, making it possible to study long-term effects of cytokines or hormones, or *in vitro* effects of molecular strategies such as interference RNA. (iv) Finally, PCLS provide the opportunity to examine the same experimental model in different species. This seems of particular importance at a time when differences between different asthma models and also between asthma models and human asthma are increasingly being recognized as a major impediment for drug development (18;19).

Given the reported similarities between GP and human airway responses, PCLS from GP would be a valuable tool in airway pharmacology, but their preparation and properties have not been reported so far. There are two studies from Shi *et al*. who used razor-cut lung explants (diameter 1 mm) and reported vascular responses to histamine and serotonin (20;21). The absence of airway data in these studies presumably relates to the practical problems of

obtaining unobstructed airways in GPs. As previously reported in the isolated perfused GP lung (22), there is a *post mortem* airway constriction that invariably occurs unless specific measures are taken. In the present communication we describe a method for preparation of PCLS from GP, show that these PCLS stay viable for at least three days and demonstrate that the airways and vessels respond adequately to various mediators and to allergen once the *post mortem* constriction has been eliminated by specific precautions during the preparation procedure. Our findings allow direct comparisons of airway responses of guinea pigs to those from rats or humans in the same model.

Materials and Methods

Animals and Material. Female Dunken Hartley guinea pigs (350g ± 30g) were obtained from Charles River (Germany). All agonists and antagonists were purchased from Sigma Aldrich (Steinheim, Germany), except leukotriene (LT) D_4 from Biomol (Hamburg, Germany), endothelin-1 from Bachem, (Weil, Germany) and U-46619 from Cayman Chemicals (Ann Arbor, MI, USA). The animal experiments and access to human lung material were approved by the local ethic committee.

Precision-cut lung slices. Guinea pig PCLS were prepared as described for other species (rat, mouse) (1;15)with the following modifications. After injection of pentobarbital (Narcoren, Pharmazeutische Handelsgesellschaft mbH, Garbsen) (95 mg/kg) the trachea was cannulated and the animals exsanguinated by cutting the *vena cava inferior*. Through the cannula the lung was filled with a low-melting point agarose solution (0.75%, final concentration) containing isoproterenol (1 μM). In order to solidify the agarose and harden them for cutting, the lungs were placed on ice for 10 min. The lobes were separated and tissue cores prepared with a rotating sharpened metal tube (diameter 8 mm). These cores were cut into 220 μm thick slices with a Krumdieck tissue slicer (Alabama Research and Development, Al, USA). Human PCLS were prepared as recently described (9).

Culture medium. The slices were incubated at 37° C in a humid atmosphere in minimal essential medium (pH 7.2) composed of $CaCl_2$ (1.8 mM), $MgSO_4$ (0.8 mM), KCl (5.4 mM), NaCl (116.4 mM), glucose (16.7 mM); $NaHCO_3$ (26.1 mM), Hepes (25.17 mM); natrium pyruvate, amino acids; vitamins; glutamine (15) and isoproterenol (1 μM) for maximal 3h. The medium was changed every 30 min during the first two hours followed by a change every hour for the next two hours, in order to remove the agarose and cell debris from the tissue. Subsequently, medium was further supplemented with penicillin and streptomycin and changed every 24 hours.

Viability, lactate dehydrogenase (LDH). Viability of the guinea pig slices was assessed by measuring the relative amount of LDH released from the slices into the incubation medium as described before (15). Three slices per well were placed into a 24-well-plate and covered with 1 ml incubation medium. At the indicated time points, slices were lysed in 1 ml 0.2% Triton X-100 solution, homogenized (Polytron; Kinematica AG, Littau, Switzerland) and then as well as supernatant analyzed by a commercially available LDH-assay (Dimension pan; Dade Behring, Schwalbach, Germany). Viability of the slices was expressed as the ratio of LDH in the supernatant to the total LDH (sum of LDH in slices and the supernatatant).

Viability, two-photon microscopy. To visualize the viability of the guinea pig PCLS we used 2-photon microscopy in combination with the LIVE/DEAD® viability/cytotoxicity assay kit (Molecular Probes, Eugene, Oregon, USA). PCLS were incubated with 5 µM Calcein AM (acetomethylester of calcein, live staining) and 10 µM ethidium homodimer (EthD, dead staining) for 40 min and washed to remove external dye. The fluorescent dyes were excited at 800 nm with a Ti:Sa femtosecond laser (Coherent, Dieburg, Germany). The laser beam was split up into 64 individual beams (beam splitter from Trim Scope, LaVision BioTec, Bielefeld, Germany) that simultaneously excited and scanned the object on the microscope. The images were acquired using a digital camera (Imager QE, LaVision, Bielefeld, Germany). The emission of calcein AM (emission filter 500/50 nm) for the cytoplasm (live staining, green) and the EthD (emission filter 625/50) for the staining of nuclei (dead staining, blue) were recorded separately. The figure shows overlay images of both dyes. Slices were analyzed 24, 48, and 72h after preparation. To visualize the total amount of dead cells, some PCLS were treated with 1% Triton X-100 for 20min prior incubation with dyes.

Measurement and Imaging. The airways and vessels were imaged and digitized with a digital video camera as described (8). Airway or vessel area before addition of the mediators was defined as 100%. Bronchoconstriction or vasoconstriction was determined as the percentage of airway/vessel area of the initial area. For the measurements, slices with comparable airway size were selected. These slices were put on 24-well-plates and fixed with a nylon thread attached to a platinum wire, in order to avoid movement of the slice during measurement. The slices were continuously covered with 1 ml incubation medium and kept at about 37°C.

The 24-well plate was positioned on the stage of an inverted microscope. Images were recorded by analogue (JAI 2040, JAI Pulnix, Alzenau, Germany) or digital camera (IRB640, Visitron Systems, Munich, Germany). A control image was taken before addition of the mediator, frames were recorded every 30s for 5, 10 or 20 min depending on the study. In order to control maximal dilation of airways, some PCLS were incubated with salbutamol (10µM). Less than 10% of the airways were precontracted and those which are precontracted showed a maximal dilation to 113% (100=baseline).

Passive-sensitization. Guinea pig PCLS were treated and incubated overnight with 1% serum from guinea pigs, that had been actively sensitized with ovalbumin (Ova) by standard protocols (7). The next day, slices were transferred into a 24-well plate with fresh medium and put under the microscope. A control image was taken, before the allergen was added.

Mediator-induced bronchoconstríction: To compare agonists involved in the allergen-induced bronchoconstriction in GP and human PCLS, cumulative concentration-response curves of histamine (0.001 – 100 μM), serotonin (0.0001 – 10 μM), LTD4 (0.01 – 1000 nM), TP-agonist U46619 (0.01 – 1000 nM) and methacholine (0.001 – 10 μM) were established.

Pharmacological intervention studies. To determine the mediators that are involved in the allergen-induced bronchoconstriction, PCLS were preincubated for at least 10 min with the Cysteinyl leukotriene-1 ($CysLT_1$)-receptor antagonist montelukast (10 μM), the thromboxane prostanoid-receptor antagonist SQ29548 (10 μM), or the histamine-1 (H_1)-receptor antagonist triprolidine (5 μM) alone or in combination before addition of a single dose of ova (100 ng/ml).

Airway relaxation. For the relaxation experiments PCLS were contracted with Mch (methacholine, 316 nM) to 10-20% of their initial airway area. Subsequently, increasing concentrations of the β-agonist salbutamol (10 nM to 10 μM), the unspecific PDE-inhibitor IBMX (3-isobutyl-1-methylxanthine, 10 nM to 10 μM) and the combination of both were added in the presence of Mch.

Statistics. Percentage data (bronchoconstriction) were transformed by the arcsin-transformation prior to analysis. The data were analyzed by two-sided t-tests or 3-way factorial mixed model analysis as indicated (JMP 5.1, Cary, IL, USA). The p-values were corrected for multiple comparisons according to the false-discovery rate procedure using the "R" statistical package. The concentration-response curves were analyzed as sigmoidal dose-response curves in Prism (Version 4.02, Graphpad Software, San Diego, CA, USA).

Results

Applied to guinea pig lungs, standard preparation protocols established for rat or mouse slices always produced PCLS in which the airways closed almost completely within the first 10 min after preparation, whereupon they did not re-open for hours. Attempts to prevent this *post-mortem* bronchoconstriction by incubation and prolonged washing in the presence of the muscarinic-receptor antagonist atropine, the TP-receptor antagonist SQ29548, the $CysLT_1$-receptor antagonist montelukast or the H_1-receptor antagonist triprolidine failed, as well as addition of a cocktail of all inhibitors (data not shown). In contrast, inclusion of the β-agonist isoproterenol (1 μM) already in the agarose medium as well as in the medium used for cutting and washing completely prevented the *post mortem* constrictions. This procedure allowed us to obtain up to 30 slices with open airways from a single guinea pig lung. After the first three hours of washings, isoproterenol exposure was discontinued, but the airways nevertheless remained open and responsive. We also examined the use of the selective β2-agonist salbutamol , but the drug was not suitable because of a longer duration of action in this preparation.

Viability of guinea pig PCLS was determined by measurement of the release of LDH into the medium (Fig. 1) and by two photon microscopy over the period of three days (Fig. 2). LDH leakage remained below 6% during this time when the medium was changed every 24h, and was 17% when the medium remained the same for 72h. Microscopic determination of viable cells showed less than 10% dead cells in the second or third cell layer (Fig 2A to D, the number of all nuclei are visible in Fig. 2D). The first cell layer was partly disrupted by the slicing procedure (data not shown). The number of dead cells remained stable over a period of 72h indicating again that a daily medium change is effective in keeping slices viable for at least three days.

Next we examined how the airways from GP and human PCLS responded to mediators relevant to asthma (Table1, Fig. 3). In GP, the biogenic amines serotonin and histamine caused bronchoconstriction with EC_{50} values of 69 nM and 217 nM, respectively, while in humans only histamine (EC_{50} 2.7μM) was effective, but not serotonin. In the GP PCLS the histamine-induced airway constriction was unaltered by the TP-receptor antagonist SQ29548 (10 μM) supporting a predominantely direct effect of histamine in this preparation (data not shown). Methacholine, the stable acetylcholine derivative, contracted airways with nearly identical EC_{50} values (about 230 nM) in GP and humans. The most potent bronchoconstrictors were eicosanoids, i.e. the stable TP receptor agonist U-46619 (GP EC_{50} 16 nM; human EC_{50}

1.3 nM) and leukotriene D_4 (GP EC_{50} 1.8 nM; human EC_{50} 5.0 nM). In GP PCLS PGD_2 was somewhat less potent with an EC_{50} of 175 nM (data not shown).

To assess whether an allergen-induced bronchoconstriction could be evoked in this new model, PCLS were passively sensitized overnight with serum from ovalbumin-sensitized GP and subsequently exposed to the allergen. Addition of ovalbumin to passively-sensitized slices caused a concentration-dependent bronchoconstriction with an EC_{50} for ovalbumin of 3.8 ng/mL (Fig. 4).

In order to analyze the mediators responsible for the allergen-induced bronchoconstriction in this new model, PCLS were pre-incubated with three different inhibitors: the H_1-receptor antagonist triprolidine (5μM), the thromboxane-prostanoid receptor antagonist SQ 29548 (10μM) and the $CysLT_1$ receptor antagonist montelukast (1μM). These inhibitors were used alone and in all possible combinations, giving a full factorial design (Fig. 4B). Pre-treatment with triprolidine or SQ 29548 attenuated the allergen-induced bronchoconstriction, and the effects of these two inhibitors were additive. Montelukast was without effect when given alone, but was effective in combination with tripolidine.

Next we examined two commonly used bronchodilators, the β_2-agonist salbutamol and the unspecific phosphodiesterase (PDE)-inhibitor IBMX. After pre-contracting airways with Mch to 10% - 20% of their initial airway area, salbutamol, IBMX or a combination of both were added cumulatively. These interventions produced a concentration-related reversal of MCh-induced contractions with significant effects of salbutamol above 100 nM and IBMX at 10 μM (Fig. 5). The combination of both bronchodilators showed a synergistic effect and was effective above concentrations of 100 nM of each drug (Fig 5C).

A particularly useful feature of PCLS is the possibility to measure both airway and vascular responses. This is illustrated in Figure 6 showing the response of an airway, a pulmonary artery and a pulmonary vein to endothelin-1. Pulmonary artery and vein can be distinguished by their relative position to the airway and by the amount of smooth muscle. The EC_{50} values for the endothelin-1-induced contraction of airways, pulmonary artery and pulmonary vein were 9.6 nM, 37 nM, and 10 nM, respectively. As previously observed in slices also from other species (23) as well as in perfused lung models (24) there was some perivascular edema around the pulmonary artery.

Discussion

For pharmacological and mechanistic studies relevant to human disease, differences in airway pharmacology between rodent models and human lungs remain an important problem. Because the preparation of PCLS is essentially the same in all species, this model provides an excellent way to compare the airway pharmacology of different species. PCLS allow investigation of single airways and vessels under cell culture conditions, and have already been used to elucidate important mechanisms of airway contraction in rat, mouse and human peripheral lungs (1;8;15;16). Here we show that PCLS from guinea-pig slices resemble human airways fairly well in terms of both mediator and allergic airways responses, and are therefore more suitable for studies relevant to human airway pharmacology than are rodent PCLS.

In this communication, we describe for the first time the preparation and the properties of guinea pigs PCLS. It is difficult to produce good quality slices from guinea-pigs because of *post mortem* bronchoconstriction that has been documented radiographically (25) and in isolated lungs (26). This constriction has been attributed to release of substance P from sensory nerves (that relaxes vessels at the same time), but other mediators or direct effects of changing microenvironment (pH, CO_2) on airway muscle may also be involved (22). Chronic treatment with capsaicin to empty substance P stores from sensory nerves or *in vivo* pre-treatment with morphine avoided the *post mortem* bronchoconstriction (27). However such treatments are impractical for routine use, carry some ethical concerns as capsaicin treatment is painful, and may interfere with the purpose of the experiments. Here we report the successful elimination of the *post-mortem* bronchoconstriction by the brief initial (3h) inclusion of isoproterenol in all the media used for preparation of the PCLS. For the same purpose, isoproterenol is also routinely used during the preparation of isolated perfused guinea pig lungs (28). Once the preparation of the PCLS is finished, isoproterenol treatment can be discontinued, whereupon airways and vessels respond normally to various stimuli showing that the effect of isoproterenol is not sustained. This is supported by the expected bronchorelaxant effects observed with addition of salbutamol to precontracted, but not to untreated preparations.

Compared to classical models of studying airway functions *in vitro* such as tracheal rings or parenchymal strips, PCLS offer many advantages such as economic use of expensive agents, longevity and, in particular, the possibility to simultaneously study airway and vascular responses; PCLS even permit to differentiate between pulmonary arteries and pulmonary veins in the same slice. This was demonstrated in the present study for endothelin-1, and in

related studies using lung explants for histamine and serotonin (20;21). In all these studies pulmonary veins responded stronger than pulmonary arteries, corroborating many other findings in rat, sheep and human lungs (29).

Guinea pigs are frequently employed for studies in pulmonary pharmacology, because they are thought to possess a pharmacological profile (6;7;30) similar to that observed in humans (31;32). However, this assumption has never been thoroughly tested in the same laboratory using the same methodology. Now having at hand both GP and human PCLS we were able to check this assumption and to define similarities and differences between both species relevant to human asthma. Comparison between GP and human airways was performed by examining their airways' responsiveness towards a variety of endogenous mediators (Table 1) and – following passive sensitization – towards allergen (Table 2). All guinea pig data and the human data are derived from the present study, whereas the allergen-induced bronchoconstriction data summarized in table 1 for human and rat PCLS was taken from previous studies (8;9).

The EC_{50} values for human and GP bronchoconstriction are almost identical for LTD_4 (leukotriene D_4) and methacholine. Airways of both species responded to thromboxane and histamine, with human airways being more sensitive to thromboxane, and GP airways being more sensitive to histamine. The most significant difference was obtained for serotonin which was quite effective in GP and completely ineffective in humans. In general, GP airways narrowed to a greater extent than human airways (Table 1, Fig. 3). The reason for this is not clear, but may, at least in part, relate to age differences tissue of (young healthy guinea pigs vs lung tissue from middle-aged adults undergoing pulmectomy). Of note, the human airways were all from small airways (diameter <2 mm), and thus free of cartilage. Overall, the comparison between GP and human airways showed that GP are not a perfect match but do nevertheless provide a reasonable agreement to humans. This is certainly true in comparison to mouse and rat airways that do not or only weakly respond to leukotrienes and histamine (1;8), both of which mediators are documented to play a role in human asthma (32).

With respect to the allergen-induced bronchoconstriction, the similarity between humans and GP on one side, and rats on the other side became even more evident (Table 2). In both GP and humans (9) both thromboxane and leukotrienes contribute to the allergen-induced bronchoconstriction. The major difference relates to histamine which is clearly more important in guinea pigs. However, none of these mediators plays a role in rats, where the allergen-induced bronchoconstriction is almost exclusively mediated by serotonin (8). In the

mouse, the mechanism of the allergen-induced bronchoconstriction appears to be even more different, and apparently mast cell independent (33).

Thromboxane, leukotrienes and histamine (at least in GP) are all potent bronchoconstrictors, and therefore it is expected that blockade of one mediator at a time will have only a small effect. And this was indeed our finding, even though significant protection could be obtained with the anti-histamine alone. The combination of the anti-histamine with either TP or cysteinyl-leukotriene antagonists was particularly effective. In general, these results are in line with previous findings in other guinea pig preparations such as the perfused lung (7), isolated tracheas (34) or parenchymal strips (30), that also show that histamine, leukotrienes and cyclooxygenase products contribute additively to the allergen-induced bronchoconstriction. Those studies, however, employed cyclooxygenase inhibitors rather than TP-receptor antagonists; in comparison, it appears that when given alone cyclooxygenase inhibitors are more effective than TP-receptor antagonist. This may indicate that other cyclooxygenase products such as PGE_2 (acting at contractile $EP_{1/3}$ receptors) and $PGF_{2\alpha}$ also are involved in the allergen-induced bronchoconstriction. It is therefore possible that in those studies some cyclooxygenase products came from infiltrated eosinophils and other inflammatory cells. Nonetheless, all these experimental studies show that the allergen-induced bronchoconstriction can only be significantly prevented if at least two of three bronchoactive mast cell mediators are blocked. In atopic subjects with asthma, anti-leukotriene drugs effectively inhibit both the early and late phase of allergen-induced bronchoconstriction (7;30). This may seem to contrast with our current inability to find a pronounced effect of the CysLT1 receptor antagonist montelukast alone in our model. However, the airway size examined in the bronchoprovocations studies in vivo (FEV1) are mainly central airways, whereas the PCLS and parenchymal strips focus on peripheral airways (7). The relative contribution of the individual mediators may well differ along the airway tree and related to differences in amounts of released mediators, airway geometry and expression of receptors. Again, when all three classes are blocked, there is complete protection. (7;9;30;32;35)

In conclusion, this is the first study on PCLS from guinea pig lungs. The slices are viable for at least three days and respond to mediators and allergens in a characteristic fashion and similar to human tissue. Although responses in the guinea-pig airways displayed some differences compared with the human tissue, the similarities appear greater than the differences. Taken together, the study shows that with respect to airway pharmacology guinea-pig PCLS are the model that so far most closely resembles that of the human situation.

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Tables

Agent	PCLS GP pD_{50}±SE [M]	PCLS GP EC_{50} [nM]	PCLS Human pD_{50}±SE [M]	PCLS Human EC_{50} [nM]
LTD_4	8.7 ± 0.10	1.8	8.3 ± 0.10	5.0
U46619	7.8 ± 0.07	16	8.8± 0.21	1.3
serotonin	7.2 ± 0.02	69	n.r	n.r.
histamine	6.7 ± 0.07	217	5.6 ± 0.22	2710
methacholine	6.6 ± 0.10	231	6.6 ± 0.10	234

Table 1: Median effective concentrations (EC_{50}) for agonist-induced airway contractions in different *in vitro* models

	Rat	Human	Guinea pig
Serotonin	X	-	-
Histamin	-	(X)	X
Thromboxane	-	X	X
Leukotrienes	-	X	X

Table 2: Mediators involved in the allergen-induced bronchoconstriction of PCLS from different species. Allergen-induced bronchoconstriction data from rat (Wohlsen et. al. 2001) and human (Wohlsen et. al. 2003) PCLS, Effective; (X), partially effective, -, non effective.

Figures:

Figure 1. LDH-release in precision-cut lung slices (PCLS). PCLS were cultured for 72 h in MEM with (open bars, n=4) or without (filled bars, n=4) medium change every 24h. *, $p < 0.01$, two-sided t-test. Data are mean ± SE.

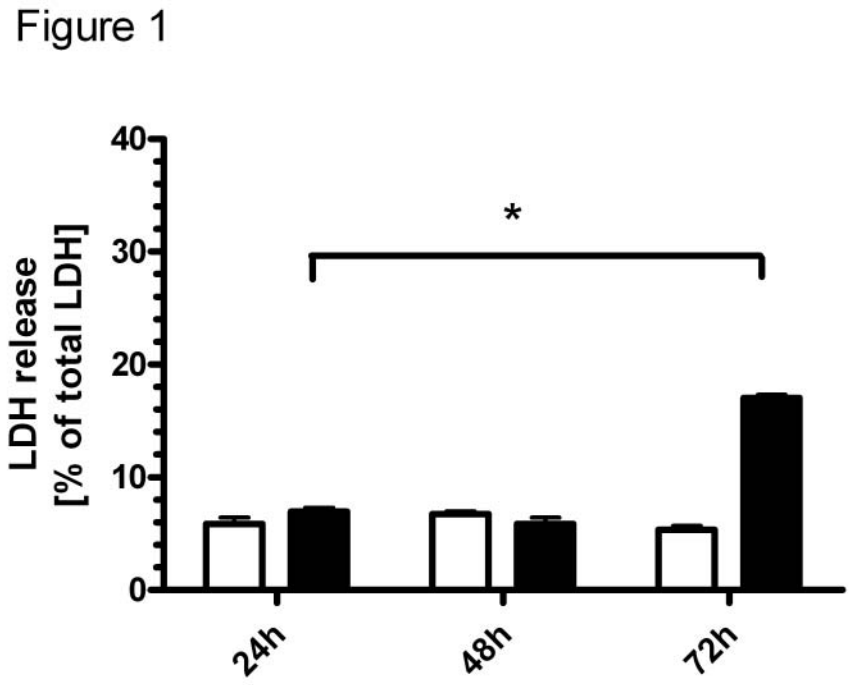


Figure 2. Two-photon microscopic image of guinea pig PCLS stained with calcein AM and ethidium homodimer. A 220 µm thin PCLS was loaded with 5 µM calcein/10 µM EthD-1 for 40 min and excited at 800 nm with a femtosecond laser. The images shows overlay frames (cytoplasm (green) and nucli (blue)). Emission was selected by the filters 500/50 and 625/50 for calcein and EthD-1, respectively. Images A to C show a viable PCLS at 24h (A), 48h (B), and 72h (C). Image D captures a PCLS pretreated with 1% Triton X-100 for 20 min followed by the LIVE/DEAD® staining. The percentage of nuclei in image A, B, or C compared to the dead slice (image D) was, 8.6%, 4.9%, or 5.5%, respectively. Bar corresponds to 80µm.

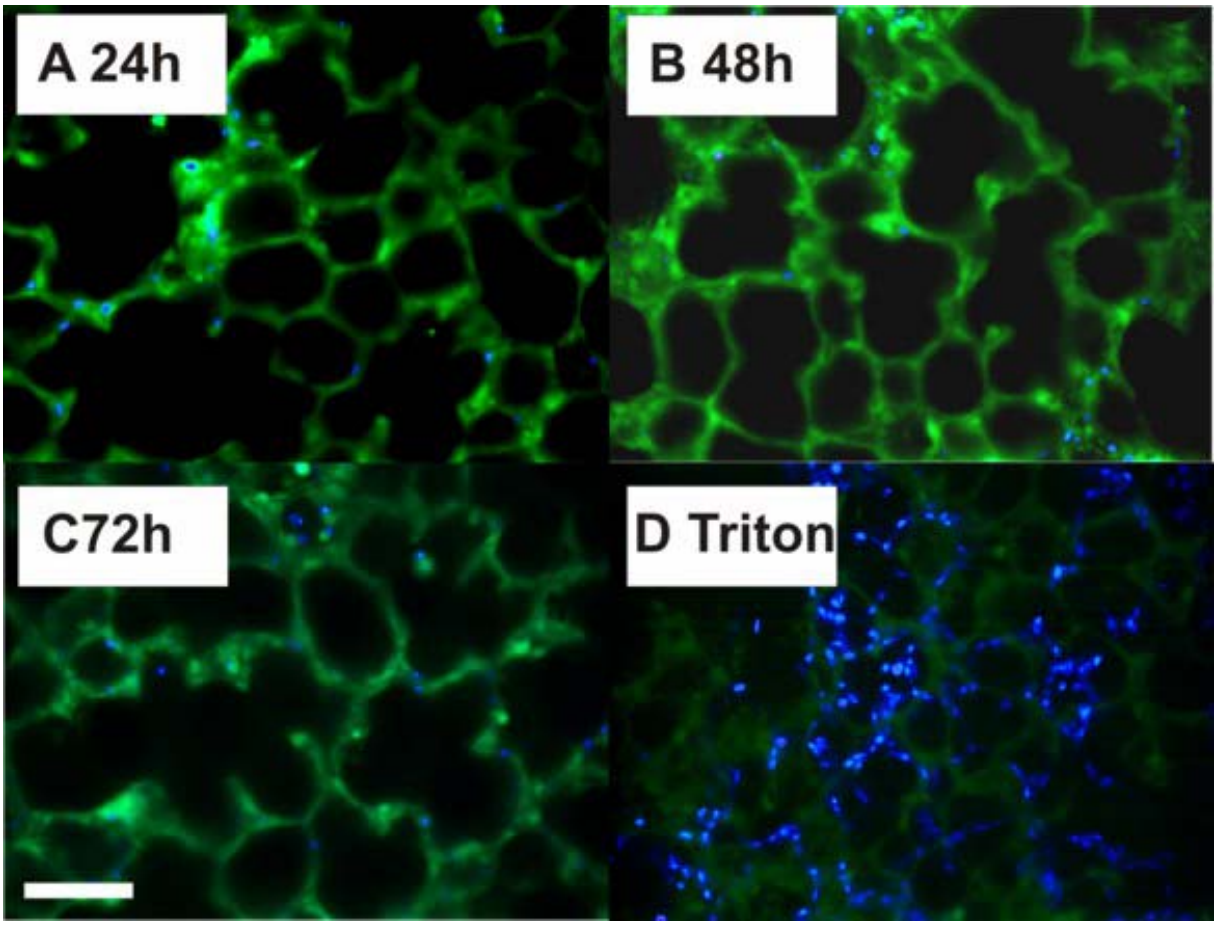


Figure 3. Bronchoconstriction in guinea pig (●) and human (■) PCLS induced by different mediators. Concentration-response-curves for (**A**) serotonin (●, n= 13, EC_{50}= 69 nM; ■, n=4, no concentration-response-curve), (**B**) histamine (●, n= 4, EC_{50}=; 217 nM ■, n= 5, EC_{50}= 2.7 µM), (**C**) leukotriene D_4 (●, n= 9, EC_{50}= 1.8 nM; ■, n= 5, EC_{50}= 5.0 nM), (D) U46619. (●, n= 4, EC_{50}= 16 nM; ■, n= 5, EC_{50}= 1.3 nM) and (E) methacholine (▼, n= 6, EC_{50}= 231 nM; ■, n= 5, EC_{50}= 234 nM,). The airway generation used for the measurement was 5 to 8 for the guinea pig and 12 to 16 for human lungs. Data are mean ± SE.

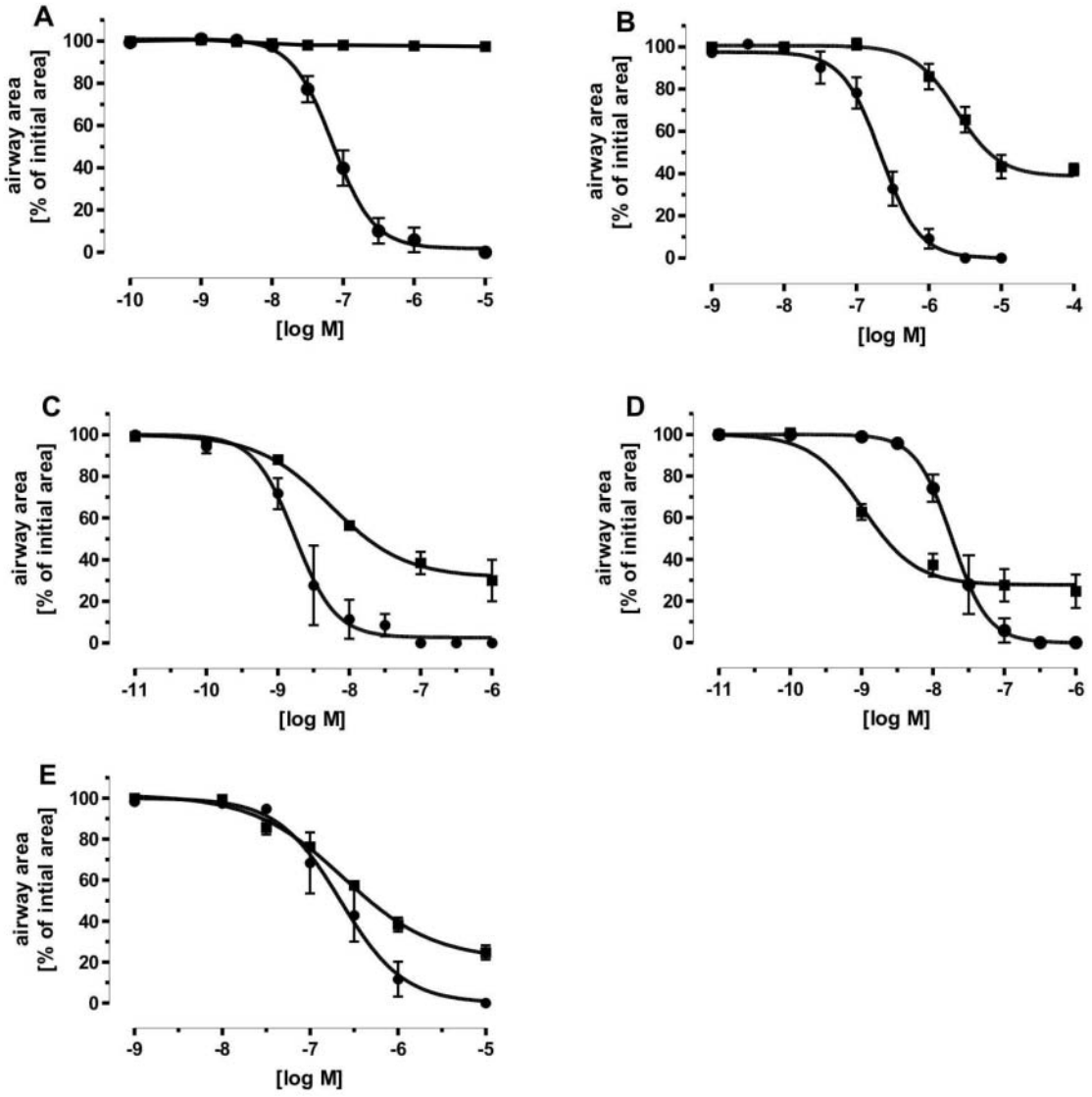


Figure 4. Ovalbumin-induced bronchoconstriction in sensitized PCLS. (**A**) Cumulative concentration-response-curve in passive sensitized (●) and non sensitized (■) PCLS; EC_{50} was 3.8 ng/mL. Similar results were obtained if each ovalbumin concentration was tested in a separate slice (data not shown). (**B**). Pharmacological inhibition of allergen-induced bronchoconstriction**.** Shown are the effects of receptor antagonists for the H_1-receptor (5 µM triprolidine, Tripro), the thromboxane-prostanoid receptor antagonist (10 µM SQ29845, SQ) and the $CysLT_1$-receptor antagonist (10µM montelukast, Monte) alone or in combination. The data were analyzed by mixed model analysis with inhibitors as fixed factors and subjects (guinea pigs) as random factor; subsequently individual contrasts were calculated and corrected by the fdr-procedure: *, $p < 0.01$, vs. solvent control. §, $p < 0.01$, vs. SQ. #, $p < 0.01$, vs. Monte. $, $p < 0.01$, vs. Tripro. &, $p < 0.01$, vs. all three inhibitors. *.Initial airway

area = 277481±51272 μm^2. Data are mean ± SE, the number of independent experiments is shown in the bars.

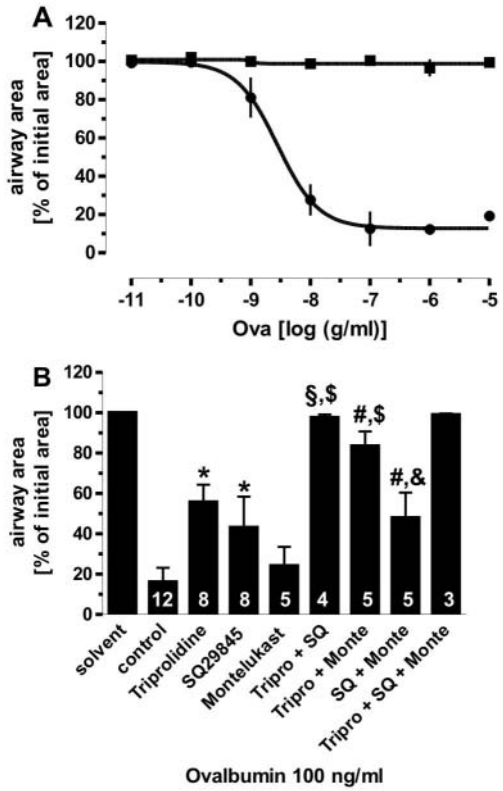


Figure 5. Relaxation of precontracted airways. Methacholine-induced (316 nM) airway contraction was reversed by (**A**) salbutamol (n=6), (**B**) IBMX (n=6) and (**C**) the combination of both (n=4). Initial airway area = 225366±40434 μm^2. Data are mean ± SE. *, $p<0.05$ vs. Mch alone.

Figure 5

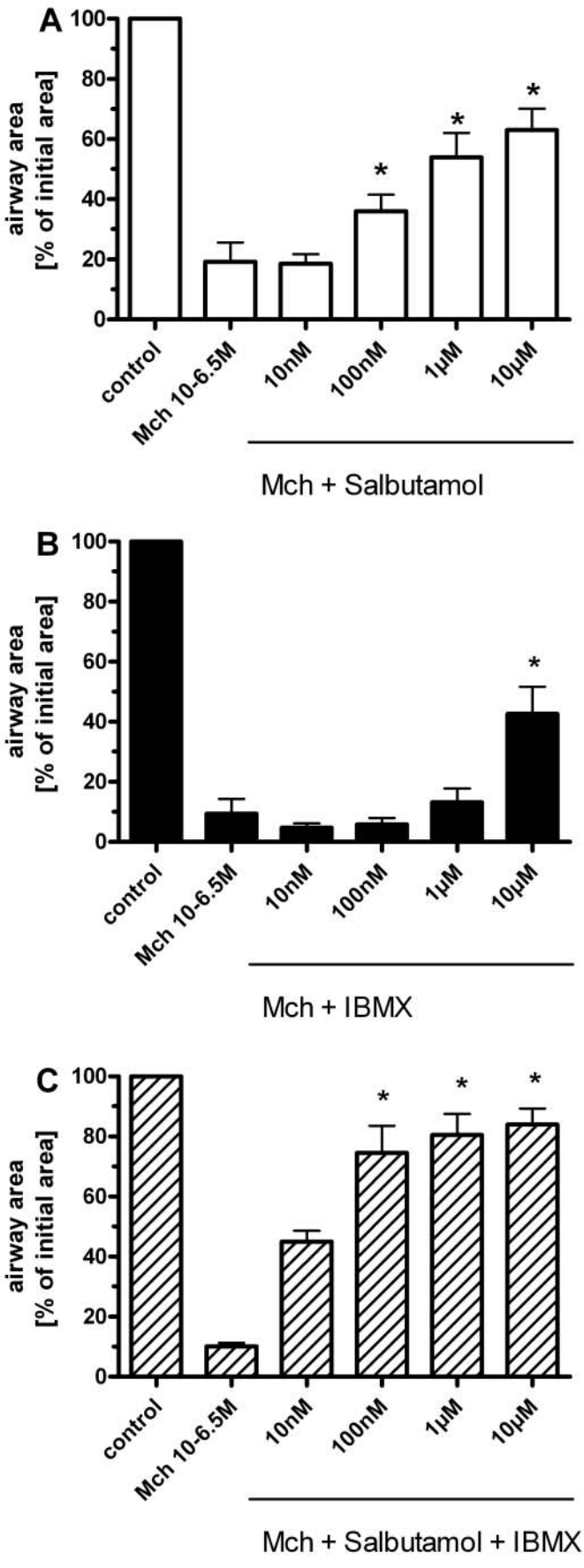
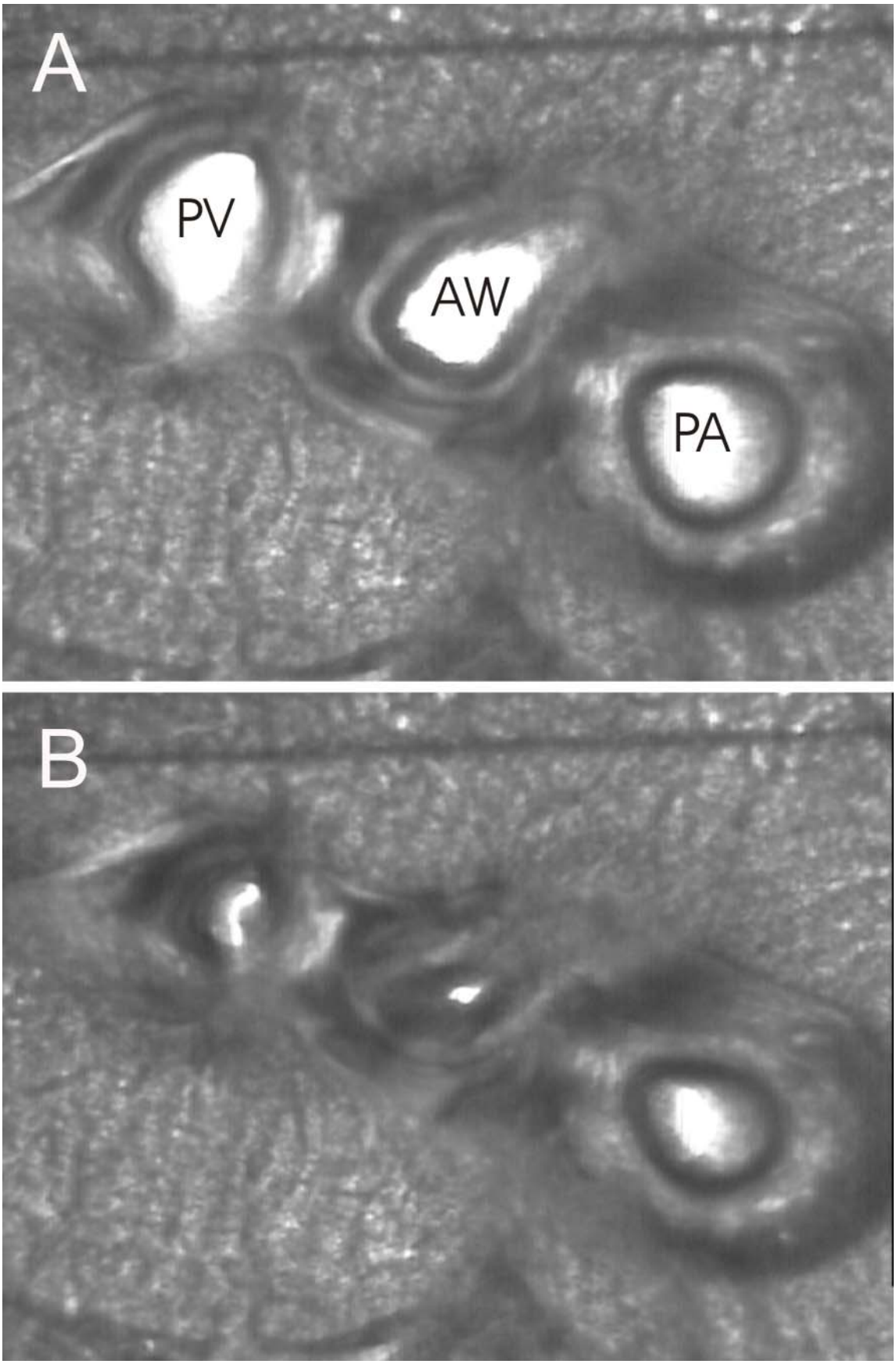
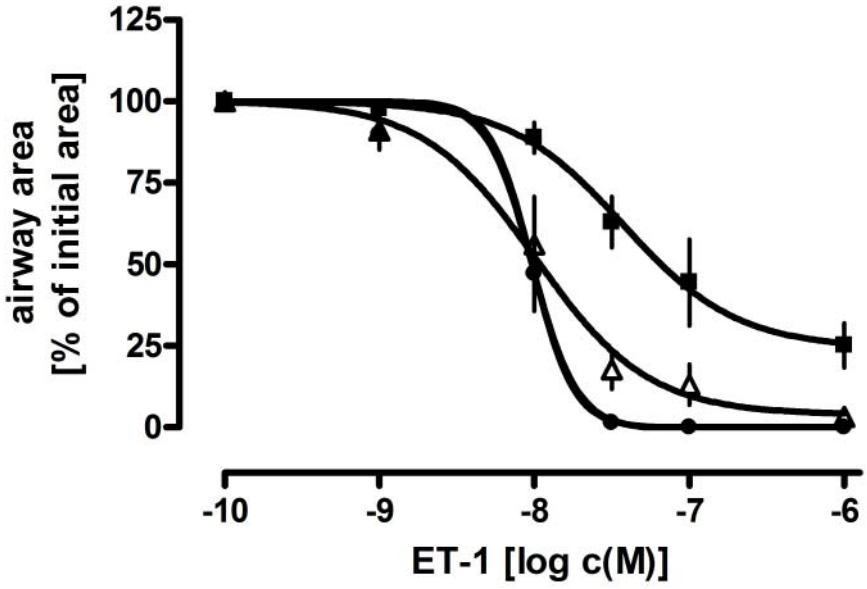

A
B
C
airway area
[% of initial area]
100
80
60
40
20
0
control
Mch 10-6.5M
10nM
100nM
1µM
10µM
*
Mch + Salbutamol
Mch + IBMX
Mch + Salbutamol + IBMX

Figure 6. Endothelin-1 (ET-1) induced contraction of airways and vessels. Microscopic image of a PCLS before (A) and after (B) exposure to endothelin-1 (AW, airway; PA, pulmonary artery; PV, pulmonary vein). **C:** Concentration- response-curve of ET-1 (0.1 nM to 1 µM) Contraction of airways (●, n= 4, EC_{50}=9.6 nM), pulmonary arteries (■, n= 5, EC_{50}= 37 nM) and pulmonary vein (▲, n= 5, EC_{50}= 10 nM) was measured in the same slice. For each concentration a new PCLS was used. Initial airway area = 231235±72611 μm^2 .Data are mean ± SE.


A
PV
AW
PA
B

C


125
100
75
50
25
0
airway area
[% of initial area]
-10
-9
-8
-7
-6
ET-1 [log c(M)]