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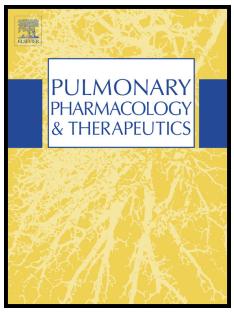

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Vasoconstriction after inhalation of budesonide: a study in the isolated and perfused rat lung

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Abstract: Clinical studies have shown that inhaled corticosteroids can induce rapid vasoconstriction in the airways, leading to decreased mucosal blood flow. The aim of this study was to investigate whether vasoconstriction of the pulmonary circulation after short inhalation of a corticosteroid can be detected in the isolated and perfused rat lung (IPL) – a model which could serve as a substitute or a complement to clinical models.

Methods: IPLs were briefly exposed to dry powder aerosol of budesonide. The pulmonary perfusate flow rate was assessed during 100 minutes post exposure. A reduction in perfusion flow rate was interpreted as vasoconstriction.

Main Results: Vasoconstriction was more pronounced after brief inhalation of 10 and 50 μg budesonide than 2 μg. The onset of vasoconstriction became statistically significant within 10-40 minutes after inhalation. Co-administration of a selective α_1-adrenoceptor antagonist (prazosin 50 nM added to the perfusate) reduced vasoconstriction by approximately 50% during 100 minutes of perfusion (p=0.003).

Conclusions: Inhaled budesonide rapidly induces pulmonary vasoconstriction suggesting a non-genomic mechanism probably related to disposition of noradrenaline at the neuro-muscular junction. This *ex vivo* model could serve as a substitute or a complement to clinical models for investigating rapid effects of glucocorticoid receptor agonists on the pulmonary/bronchial circulation.

Word count abstract: 202

Keywords; corticosteroids, budesonide, nongenomic, pulmonary circulation, vasoconstriction

1 Introduction

Corticosteroids applied topically on the skin give rise to a blanched area due to vasoconstriction. Skin blanching tests have therefore frequently been used for screening of potency of corticosteroids in drug development [1, 2]. Inhaled corticosteroids (ICS) have prominent effects on inflammatory changes of the tracheobronchial airway vasculature in patients with bronchial asthma. ICS are known to exert their effect on airway vasculature through a delayed genomic [3] and a non-genomic phenomena, in common with topical steroids, thereby causing significant vasoconstriction in the larger airways and modifying, at least in part, some of the pathophysiological components of airway narrowing in bronchial asthma [4]. These clinically observed effects include suppression of proinflammatory responses such as hyperperfusion, microvasculature hyperpermeability, oedema formation, inflammatory cell recruitment and the formation of new blood vessels [5, 6]. Inhaled fluticasone propionate (FP) has been shown to reduce blood flow in airway mucosa of asthmatics and healthy volunteers [1]. Vasoconstrictive effect of ICS has also been demonstrated by inhalation of beclomethasone dipropionate and budesonide [4]. High doses of ICS have shown to significantly improve lung function in patients with asthma within 1-2 h of treatment. This rapid improvement leading to an improved discharge rate of asthma patients from the emergency rooms suggests that a rapid nongenomic mechanism of ICS is involved [6]. The commonly accepted genomic mode-of-action for corticosteroids involves the formation of a cytosolic corticosteroid-receptor complex that is subsequently translocated to the nucleus for its mode of action [3]. However, few studies have brought mechanistic insight to the rapid non-genomic action of corticosteroids. Iversen was first to demonstrate a role in vasomotor tone for corticosteroid-sensitive extraneuronal uptake of noradrenaline, mediated through saturable membrane transporters [7, 8]. Recently, pretreatment

with the selective α_1-adrenoceptor antagonist terazosin was suggested to abolish the effect of fluticasone propionate on mucosal blood flow. This would indicate that noradrenergic signalling was involved in the corticosteroid sensitive regulation of smooth muscle tone in airway vasculature [9, 10].

The isolated and perfused rat lung (IPL) has been used in our laboratory as a model for studying effects of various pharmacological interventions on lung mechanics and vasoconstriction [11, 12]. Recently, the DustGun aerosol technology has been used to expose the IPL, via dry powder inhalation, to aerosols of drugs and toxicants [13-15].

In the present study we combined these two techniques to achieve three specific aims; i) to establish a method for accurately measuring the perfusate flow rate of the rat IPL perfused in the single-pass mode at a constant hydrostatic pressure, ii) to measure the perfusion flow rate for up to 100 minutes after short inhalation exposures of the IPL to air control, lactose and budesonide, iii) to study the influence of noradrenergic selective antagonist on budesonide-exposed lungs.

2 Material and methods

2.1 Isolated perfused lungs

The experiments were approved by the local animal ethical committee. Thirty female Sprague-Dawley rats weighing 285-310 g were used in this study. The surgical procedure and lung mechanical measurements were performed as described in detail elsewhere [13]. The lungs were perfused with a 2% bovine serum albumin Krebs-Ringer physiological buffer. Briefly, each animal was anesthetised by injecting pentabarbital intraperitoneally and the chest was opened. A perfusate inlet catheter was attached to the pulmonary artery and perfusate was exiting the lung

through a left heart catheter. A tracheoctomy was perfomed and lungs and heart were dissected *en bloc* and placed in an artificial thoracic chamber. A negative pressure of 0.5 kPa was applied in the chamber and lungs were ventilated at 75 breaths/min, and thus, an alternating negative pressure was established inside the chamber. Lung mechanical data was collected throughout the experiment using an EMKA system for monitoring the IPL (EMKA technologies, Paris, France). The following lung mechanics were measured at a baseline: conductance (G_{aw}) = 66 ± 21 mL/s/kPa, dynamic compliance (C_{dyn}) = 2.7 ± 0.4 mL/kPa, and tidal volume (TV) = 1.2 ± 0.2 mL; (SD, n = 30).

2.2 Measurement of perfusion flow rate

Criteria for constant hydrostatic pressure perfusion; i) the vertical distance between the free liquid table in the perfusate reservoir and the apex of the heart of the IPL inside the thoracic chamber was set to approximately 100 mm H_2O, equal to a hydrostatic pressure of 1.0 kPa, ii) the peristaltic pump speed was adjusted so that the reservoir was continually overflowed with perfusate, in order to keep the free liquid table at a constant level. Single-pass perfusion of the IPL was used. The perfusate flow rate was repeatedly measured five times per minute for up to 120 minutes, providing 600 discrete data points per exposure. The exposure cycle of the DustGun aerosol device was synchronized to allow for a pre-exposure flow measurement period at baseline (15). The exposures were made during the 95^{th}-100^{th} flow rate measurements. At the 600^{th} sample the data acquisition was terminated. The flow rate was measured by collecting the perfusate in a container placed directly on an electronic scale (Sartorius ED3202S-CW, Sartorius AG, Germany). By directing the flow through a 3-way valve for 4s to the sample container and for 8 s to drain, discrete weight gain intervals could be recorded by the scale. A data acquisition protocol

written in Labview (National Instruments AB, Solna, Sweden) was used to record the step-wise increase in cumulative weight of the sampled perfusate. An algorithm was designed to identify the time point and weight at the beginning and end of each weight gain interval. The pulmonary perfusion flow rate (mL/min) of each sample interval was calculated by dividing the weight gain of each sampling interval with its corresponding time duration (eqn 1).

eqn1.

$$Q_n = \frac{m_{n+1} - m_n}{\rho\ t_{sample}},\ \text{n=1…600}$$

Q_n = Pulmonary perfusion flow of the n^{th} sample (mL/min)

m_n = recorded mass at the beginning of the n^{th} interval (g)

m_{n+1} = recorded mass at the end of the n^{th} interval (g)

ρ = density of perfusate (g/mL)

t_{sample} =sample interval (min)

2.3 Short inhalation exposures of the IPL to dry powder aerosols

The characterization of aerosols generated by the DustGun apparatus and the short inhalation exposures of the IPL to respirable aerosols in high concentrations have been previously described [13-15]. In the present study the IPL was exposed to budesonide in a series of doses delivering respectively, 2, 10 or 50 microgram budesonide (BUD-2, BUD-10 and BUD-50, respectively) in different groups of lungs (Table I). Lactose is commonly used as an excipient in inhalation drugs and was used a particle aerosol control (Table I). Budesonide and lactose were provided by AstraZeneca R&D, Lund. Measurements of the perfusion flow rate in air exposed IPL preparations were used as a negative control. In each experiment an air control period was

used during which the perfusion flow rate was recorded for 20 minutes prior to any exposure. The lungs were exposed to air by the DustGun apparatus and the perfusion flow rate was sampled 100 minutes after the air control period.

2.4 Co-exposure of α_1-adrenoceptor antagonist and BUD-10

Prazosin, a selective α_1-adrenoceptor antagonist, was used to test the effect of the antagonist on pulmonary vascular tone in IPLs co-exposed to budesonide. Prazosin hydrochloride (Sigma-Aldrich Sweden AB, Stockholm, Sweden) at 50 nM was administrated to the perfusate throughout the total time of perfusion in combination with short inhalation exposures to BUD-10.

2.5 Data and Statistical evaluation

To remove noise from the flow rate measurements, the raw data was low-pass filtered by calculating the running median of a window of ten consecutive measurements (Q_n) [16]. The window was advanced with one sample for each calculation of Q_n from the first to the 600^{th} sample. The first 100 samples, corresponding to the air control period, were used to assure that a steady baseline was maintained throughout the first 20 minutes of lung perfusion. For treatments and controls, each perfusion flow rate measurement was normalized to its 20 minute low-pass filtered reading by assigning it there the value 100% ($Q_{baseline}$). The relative perfusion flow rate (q_{nlung}) from time 0-100 minutes post exposure of each sample *n* was calculated by dividing each Q_n of that series with the corresponding $Q_{baseline}$ (Eqn 2). The reduction of perfusate flow rate following treatment was analysed by dividing the period of perfusion post-exposure into three different time periods; from time zero to 10 minutes, from 10 minutes to 40 minutes, and from 40 minutes to 100 minutes. For each control and treatment, the average relative pulmonary perfusion

flow rate ($\hat{q}lung$) was calculated for the three subdivided periods of perfusion and for the whole period of perfusion post exposure. In figure 2, for the purpose of clarifiaction, $\hat{q}lung$ was expressed as a reduction in % of the average pulmonary perfusion flow rate (%*qlung),* (Eqn. 3). A two-way analysis of variance (ANOVA) was applied for comparisons between treatments (using Sigmastat ver 3.0). Pair-wise multiple comparisons were performed with a Bonferroni correction for treatment, time and the effect parameter $\hat{q}lung$. The differences were regarded statistically significant when the *p*-value was less than 0.05. Values are presented as mean±SD.

Eqn. 2

$$q_{nlung} = \frac{Q_n}{Q_{baseline}} \quad \text{for } Q_n \; 1\ldots500$$

q_{nlung} = relative perfusion flow rate of the n^{th} sample

$Q_{baseline}$ = baseline perfusion flow rate at 20 minutes (mL/min)

Q_n = perfusion flow rate of n^{th} sample (mL/min)

Eqn. 3

$$\%qlung = (1 - \hat{q}lung) \times 100$$

$\hat{q}lung$ = average relative perfusion flow rate (averaged over any given interval (0-10, 10- 40, 40-100 or 0-100 minutes)

%*qlung* = percentage reduction of average relative perfusion flow rate (averaged over any given interval (0-10, 10-40, 40-100 or 0-100 minutes)

3 Results

The perfusion of the IPL, with constant hydrostatic pressure, provided a stable baseline in all experiments. The measured perfusate flow rate during the 20 minute long baseline interval was 20.0 ±1.8 mL/min (mean±SD, n=30). During ventilation of the air control lungs for 120 minutes, we observed a linear deterioration in the lung mechanics of 5-10 % for *Cdyn* and of 2-4 % for *Gaw*. This level of deterioration in lung mechanics in the IPL was also seen in lungs treated with lactose and budesonide and has also been reported in the literature [17].

The perfusate flow rate of air control lungs decreased slightly over time. At 100 minutes post exposure, the perfusate flow rate (q_{nlung}) was 94.5 ±3.7 % (mean±SD, n=5) of the baseline value, and the average perfusate flow rate ($\hat{q}lung$ **)** for the entire perfusion period was 97.9±3.3 % (mean±SD, n=5) of the baseline value (figure 1).

The mass median aerodynamic diameter (MMAD) of budesonide and lactose aerosols was 2.3 μm and 5.5 μm, respectively [15]. The predicted lung deposition in μg and the average initial concentration of BUD in the lungs immediately after exposure were estimated for each exposure group (Table I) [15].

Lactose exposures were chosen as a control for lungs exposed to a particle aerosol without apparent pharmacology, to compare with budesonide exposed lungs. The estimated deposited lung dose of lactose was 40±11 μg (mean±SD, n=5). The average flow rate of lungs exposed to lactose ($\hat{q}lung$ **)** was compared with air control lungs. None of the pair-wise comparisons of air control lungs and lactose differed significantly (table II and figure 2). At 100 minutes post exposure, the perfusate flow rate (q_{nlung}) of lactose exposed lungs was 86.6±3.6 %

(mean±SD, n=5) of the baseline value and the average perfusate flow rate ($\hat{q}lung_{0-100}$) for the entire perfusion period was 95.0 ±1.7 % (mean±SD, n=5) of the baseline value (figure 1 and 2).

After exposures of the IPL to budesonide, the average perfusate flow rate ($\hat{q}lung_{0-100}$) was measured for 100 minutes post exposure (figure 1a, b). In comparison to lactose-exposed lungs, there was a gradual reduction in $\hat{q}lung$ for BUD-10 and BUD-50 (figure 1a). The onset of reduction of perfusion flow rate became significant for BUD-10 and BUD-50 in comparison to lactose during the 10 to 40 minute period **(** $\hat{q}lung_{10-40}$ **),** (p= 0.012 and 0.049, respectively). The perfusion flow rate for the BUD-2 exposures did not differ significantly from that of the lactose exposures. Hence, these comparisons demonstrate a dose-dependent onset of the perfusate flow rate reduction (figure 2). Further, while the flow rate of the BUD-2 exposures showed no effect on overall qlung ($\hat{q}lung_{0-100}$**)**, the BUD-10 exposures substantially reduced the perfusion flow rate of the lungs. Then at the BUD-50 exposures there was a smaller reduction in $\hat{q}lung_{0-100}$, causing an unexpected bell-shaped dose-response for the budesonide sensitive pulmonary flow rate reduction. This smaller reduction for BUD-50 was as significant as noted for BUD-10 during the last 60 minutes of perfusion ($p<0.001$), but for the total period the difference in perfusion flow rate was non-significant (p= 0.185) (figure 2). The co-administration of BUD-10 with 50 nM prazosin given via the perfusate resulted in a decreased reduction of the perfusate flow rates ($\hat{q}lung$) and delayed the onset of reduction in comparison to BUD-10 (figure 1b). Within the 40 to 100 minute period, as well as for the whole time period, co-exposure to prazosin decreased significantly the flow reduction induced by the BUD-10 exposure ($p<0.001$ and =0.003, respectively). The flow reduction induced by the co-exposure of BUD-10 with prazosin 50 nM was roughly half that of the BUD-10 exposure alone (figure 2). The administration of 50 nM

prazosin hydrochloride by itself had no affect of the pulmonary perfusion flow rate. For all comparisons of BUD with the positive control (lactose), a significant reduction of perfusate flow rate compared to the positive control was interpreted as vasoconstriction of pulmonary vessels.

4 Discussion

This study, performed in the isolated and perfused rat lung, demonstrates a significant reduction in the pulmonary perfusion flow rate within 40 minutes after inhalation of sufficiently high doses of budesonide. It has been known that after administration of corticosteroids, a minimum of one to two hours are required for induction of their genomic effects, i.e. appearance of *de novo* synthesised proteins. The rapid reduction of perfusion flow rate observed in this study may be a result of a non-transcriptional and corticosteroid-sensitive disposition of noradrenaline (NA) causing vasoconstriction of pulmonary vessels [20].

Several studies in humans have addressed the effects of ICS on blood flow in the bronchial mucosa [1, 18], where data on perfusion of the bronchial tree have been derived from the measured disappearance of an inhaled inert gas from the airways (exhaled air) into the tracheo-bronchial circulation [1]. However, fewer clinical studies have addressed a possible effect of ICS on the pulmonary circulation. In asthmatics, inhalation of platelet aggregation factor (PAF) has been used for acute induction of a perfusion-ventilation mismatch in asthmatics [19]. Within 45 minutes after inhalation of fluticasone propionate, the corticosteroid ameliorated the PAF induced gas exchange mismatch, measured as improved systemic arterial oxygen tension. The authors proposed that this increase in systemic oxygen tension most likely reflected a corticosteroid-dependent effect on pulmonary circulation, leading to a better alveolar ventilation-blood perfusion balance. To our knowledge, this is the only study to show an effect of ICS on the pulmonary circulation in a clinical model.

Yet, an important question is whether the presently described results derived from measurements on the pulmonary circulation in rats have any bearings on clinical findings in the bronchial circulation as discussed above. Thus, in order to bring together our findings of rapid corticosteroid-sensitive reduction of perfusion flow rates in the IPL with corticosteroid-sensitive vasoconstriction of bronchial vessels of the human lung, the discussion will be narrowed down to the following topics: 1. mechanism for rapid vascular effects of ICS in humans; 2. viability, disposition and uptake of NA in the IPL; 3. sympathetic innervation of the pulmonary and bronchial circulation and; 4. the applied corticosteroid dose to target tissue e.g. ex vivo doses compared to clinical doses.

1. Horvath and co-workers have, in addition to clinical studies, brought deep mechanistic insight into the topic of vascular effects of ICS [4,9-10] . They have demonstrated, in isolated human bronchial arterial smooth muscle cells [20], that corticosterone, budesonide and methylprednisolone dose-dependently inhibit – by an unknown but rapid mechanism - the extraneuronal uptake of NA. By doing so, the corticosteroids increase the concentration of NA at the neuro-muscular junction that may result in increased α_1-adrenoceptor occupancy, noradrenergic signalling and vasomotor tone. This mechanism can certainly explain the acute bronchial vasoconstrictive action of inhaled ICS in humans [20].

2. Few investigators have previously studied the mechanisms of disposition of NA in the isolated and perfused rat lung. In a tracer study by Metting et al [21], where NA was administered as a bolus dose into the pulmonary artery, the authors concluded that NA is mainly retained in the lung tissues in a slow intra-cellular compartment that is sensitive to inhibition of both neuronal and extraneuronal reuptake of NA. This study not only illustrates the utility of a simple

ex vivo model compared to a much more complex *in vivo* situation, but certainly provides evidence that uptake mechanisms of NA are functional in the IPL. Furthermore, in the present study we have demonstrated that the perfusion flow rate in the IPL is sensitive to high concentrations of ICS and that the selective α_1-adrenoceptor antagonist prazosin reduced the budesonide-induced vasoconstriction by about 50%, which suggest that the effect of budesonide is mediated through the α_1-adrenoceptor [9,10]. Hence, in our model we hypothesise that the selective α_1-adrenoceptor antagonist prazosin competes with NA at the binding sites of the α_1-adrenoceptor within the lung vessels, and thus, renders the pressor effect induced by budesonide less pronounced. Thus, in the IPL, a corticosteroid-sensitive redisposition of NA in lung tissues – through an unknown mechanism - may facilitate an increase in α_1-adrenoceptor occupancy, noradrenergic signalling and vasomotor tone [20]. In future studies, we will continue to address the role of a corticosteroid-sensitive disposition of NA, in the light of both neuronal and extraneuronal uptake.

3. We surveyed the literature for a cross-species comparison of the anatomy of sympathetic nervous system, and more specifically, the distribution of nor-adrenergic fibers in bronchial and pulmonary vasculature. The bronchial vessels in rats, monkeys and humans are sustained by a network noradrenergic fibers [22-23]. Pulmonary vessels of the rat have been demonstrated by some workers to have a low frequency of noradrenergic innervation. El-Bermani [23] has reported that the branched pulmonary arteries and the small pulmonary veins with surrounding smooth muscles in Wistar rats were frequently innervated with nor-adrenergic fibers. The main flow resistance of the pulmonary circulation is considered to stem from these richly innervated plexuses of pulmonary veins. Thus, there seems to be some similarities between both the frequency and distribution of the nor-adrenergic innervation in the pulmonary veins and in the

bronchial arteries. This insight suggests that, after stimulation of noradrenergic nerve-endings, similar physiological responses such as vasoconstriction are to be expected in both types of neuro-muscular plexuses, i.e. pulmonary bronchial arteries and pulmonary veins.

4. An important aspect of comparing vasoconstriction in clinical and preclinical models is whether the applied exposures in preclinical models are relevant for the clinical situation. In humans, after inhalation of a nominal dose of 800 μg of budesonide - a dose that significantly reduced mucosal blood flow in healthy subjects as well as in asthmatics [4] - typically 20% of the nominal dose is expected to deposit in the lung [24]. If this mass is dissolved and distributed in 500 g human lung tissue, this would correspond to an average initial tissue concentration in the lung of approximately 1 μM directly after inhalation (it should be kept in mind, however, that budesonide is not likely to be homogenously distributed, and that concentrations locally in parts of the mucosa can be much higher). For comparison, we have calculated that in the rat IPL, exposures to 10 μg budesonide (that caused a rapid reduction of flow rate) results in an initial average tissue concentration of 15 μM, or about one order of magnitude higher than observed in the clinic. This difference in initial target tissue concentration is not unreasonable considering: i) there is at least a five-fold difference in airway air/blood barrier thickness between the two species (human >rat) [14], and, ii) there is most likely a more peripheral deposition of particles in the IPL setup in comparison to administration of budesonide to central airways in humans, which is likely to give a much more rapid extraction of the drug to the circulation from the rat lung than from the human lung. It is reasonable to assume that soon after inhalation, a faster extraction of higher tissue concentrations from the rat lung leads to local lung tissue concentrations similar to those of corresponding human lung exposures. Some support for this argument comes from the measured time to reach the maximum concentration (tmax) of budesonide in the blood in the two cases. For budesonide inhaled by healthy male subjects, the tmax in the systemic circulation

range from 10-24 min [25], whereas we have measured the tmax in the single-pass perfusate of the rat IPL to be 0.7-1.1 min [15]. Even if the clinical tmax is somewhat delayed because of being sampled on the venous side of the circulation, whereas the arterial side is sampled in case of the IPL, it is reasonable to assume that the thicker air/blood barriers of human lung would retain the budesonide at least one order of magnitude longer than the thinner barriers of the rat IPL. A significant reduction of the perfusion flow rate in our IPL model should therefore require a higher initial concentration than in case of clinical exposures.

Hence, studies from our laboratory and others have shown that the rat IPL can serve as model to study the disposition of noradrenaline and the corticosteroid-sensitive vasoconstriction of the lung. Therefore, it is reasonable to suggest that the effect of corticosteroid exposures on perfusion flow rates as observed in our IPL model after administration of sufficiently high doses of inhaled budesonide, is most likely controlled by the retention of noradrenaline at the neuro-muscular junction, similar to the vascular effects of ICS observed in humans and attributed to a nongenomic corticosteroid effect [1, 8, 20]. Moreover, similar noradrenergic innervations of pulmonary veins and bronchial arteries in rats and primates allow us to suggest that the rat IPL can serve as model to study corticosteroid sensitive vasoconstriction of the bronchial circulation in humans. The model can, thus, serve as a valuable substitute or complement to more laborious clinical models for investigating rapid, corticosteroid-dependent effects on the pulmonary/bronchial circulation, which may have important implications for the disposition and efficacy of these drugs in the lung, alone or in combination with other inhaled drugs [26].

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6 Tables I-II

Table I	**n**	**Mass deposited (μg)**	**Lung average conc. (μM)**
Drug			
BUD-2	5	2.5±0.3	4
BUD-10	5	11±0.8	15
BUD-50	5	48±9.3	75
BUD-10 +Prazosin	5	12±0.2	15
Lactose	5	40±11	80

Table I) The deposited mass (μg) after inhalation of budesonide and lactose in the IPL and the calculated initial average lung concentration in μM of budesonide in 1.5 g lung tissue.

Table II	**0-10 minutes**	**10-40 minutes**	**40-100 minutes**	**0-100 minutes**
Treatment	$\hat{q}lung_{0-10}$	$\hat{q}lung_{10-40}$	$\hat{q}lung_{40-100}$	$\hat{q}lung_{0-100}$
Air Control	1.0±0.02	0.99±0.03	0.97±0.04	0.98±0.03
Lactose	1.0±0.01	0.98±0.01	0.92±0.03	0.95±0.02
BUD-2	0.99±0.01	0.98±0.01	0.91±0.03	0.94±0.02
BUD-10	0.98±0.03	0.93±0.03 *	0.79±0.06 **	0.85±0.04 **
BUD-10 praz	0.99±0.01	0.97±0.02	0.87±0.02*,§§	0.91±0.02 §§
BUD-50	0.97±0.02	0.93±0.02 *	0.85±0.03 **	0.89±0.02**

Table II). The mean relative pulmonary perfusate flow rate ($\hat{q}lung$) for the different intermediate periods and for the total time of perfusion for the different treatments. The onset of vasoconstriction and the effect of prazosin (praz) on budesonide- mediated vasoconstriction was tested by 2-way ANOVA, pairwise multiple comparisons (Bonferroni t-test) * denotes significantly different from lactose, * = $p<0.05$ and ** = $p<0.01$. § denotes significantly different from BUD-10, §§= $p<0.01$. Mean ± SD n=5.

7 Legends to figures;

Figure 1a and b) Relative perfusate flow rate (*qlung*) in the lungs plotted versus time. Data shown as means from 5 experiments; a) time response in lungs exposed to air control, lactose, budesonide 2, 10 and 50 μg (BUD-2, BUD-10 and BUD-50, respectively); b) Vasoconstriction of BUD-10 without and with the addition 50 nM prazosin in perfusate.

Figure 2) Reduction of relative perfusate flow rate %*qlung* (Eqn 3) in the lungs, averaged over intermediate periods and over total perfusion time. For all treatments from left to right and as indicated in figure: air control, lactose, budesonide 2 μg (BUD-2), budesonide 10 μg (BUD-10) without and with prazosin (praz) , budesonide 50 μg (BUD-50). Data shown as mean±SD (N=5); * denotes significantly different from lactose, § denotes significantly different from BUD-10, *=$p<0.05$ and **=$p<0.01$, §§=$p<0.01$.

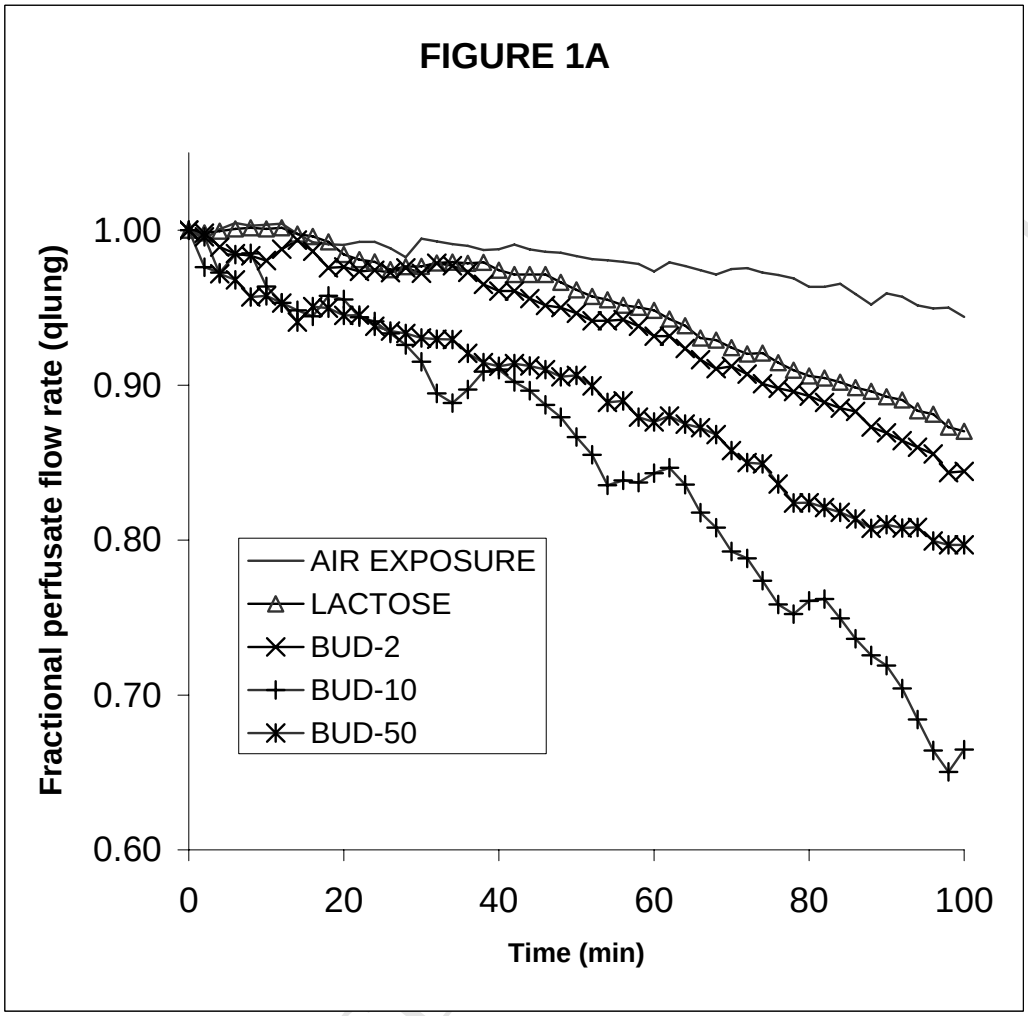
FIGURE 1A
Fractional perfusate flow rate (qlung)
1.00
0.90
0.80
0.70
0.60
0
20
40
60
80
100
Time (min)
AIR EXPOSURE
LACTOSE
BUD-2
BUD-10
BUD-50

FIGURE 1B

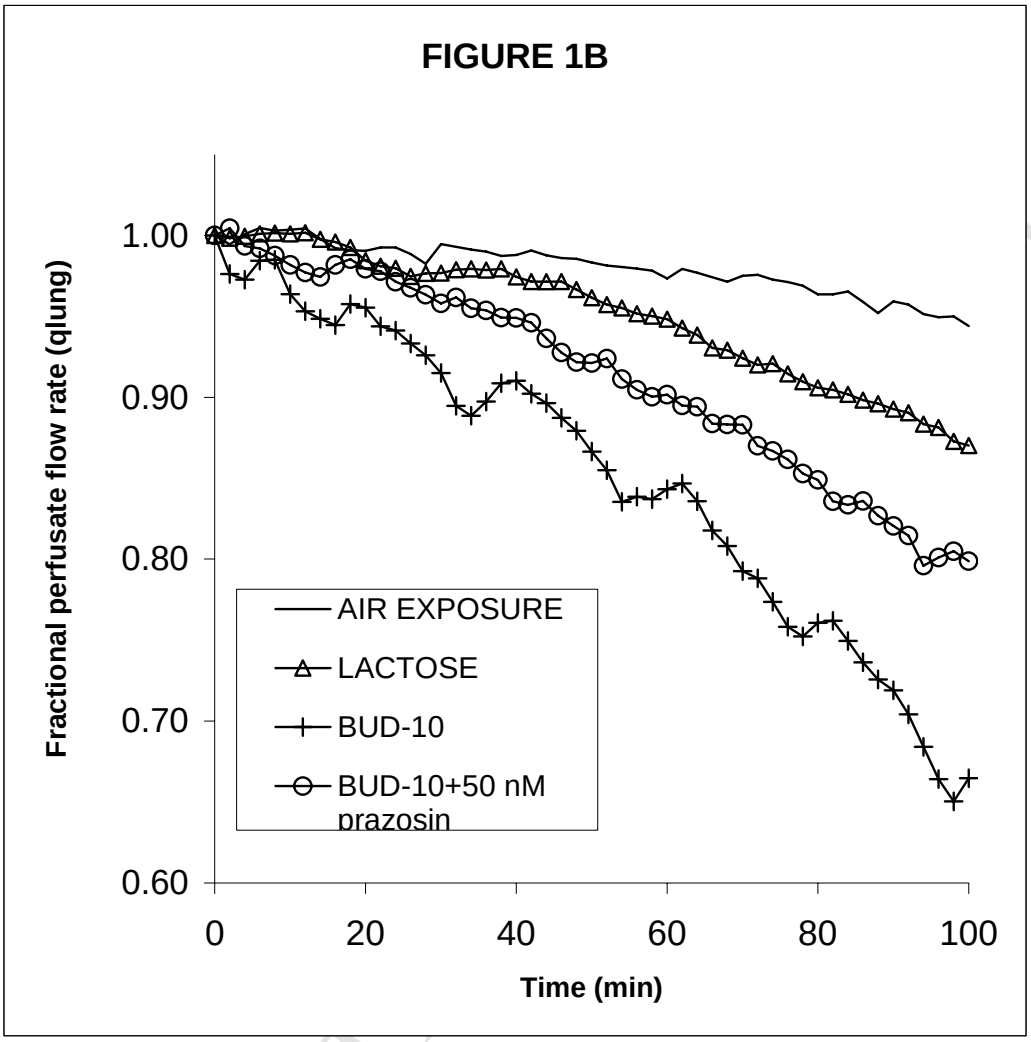

FIGURE 2