EFFECTS OF PULMONARY CONSTRICTOR AGENTS ON LUNG MECHANICS: AIRWAY AND TISSUE RESPONSES

Ph.D. Thesis

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SUMMARY

Numerous studies have established the involvement of both the airways and the lung parenchyma in pulmonary constrictor responses.

In the present thesis, the effects of three iv.-delivered pulmonary constrictor agents of pathophysiological importance (histamine, methacholine and endothelin-1) on the mechanical properties of the airways and lung tissues were studied in dogs, rats and guinea pigs.

A noninvasive assessment of the respiratory mechanics, using the low-frequency forced oscillation technique, was applied to separate the airway and tissue components. The model-based evaluation of the pulmonary input impedance data was used to characterize the airway and tissue mechanics under control conditions and during pulmonary constriction. To circumvent the problems of flow measurements in a small animal, we adopted the wave-tube technique to measure the mechanical impedance of the respiratory system in rats and guinea pigs. In agreement with previous findings in other mammals, the fitting of the impedance data to a model involving an airway and a constant-phase tissue compartment resulted in no systematic errors.

Our studies on the partitioning of the lung response into airway and parenchymal components have confirmed that the constrictor agents act on both the airway and tissue compartments. Although the differences in species (in terms of the distribution of receptors and the quantity of airway smooth muscle) and/or methodological factors may influence the pattern and the magnitude of the lung response, the constrictor agent and its delivery route seem to be the primary determinants of the shares of the airway and tissue compartments in the provoked constriction.

These studies demonstrated that different underlying mechanisms and/or sites of action were activated in response to the effects of the three pulmonary constrictor agents. Analysis of the model performance further indicated that, at high doses of all the agonists studied, the constriction of the peripheral airways was highly heterogeneous.

INTRODUCTION

Pulmonary constrictor agents – Pathophysiological overview

Histamine (HIST) and endothelin-1 (ET-1) are important inflammatory and proinflammatory mediators of pathophysiological effects in endotoxemia, sepsis, adult respiratory distress syndrome (ARDS) and asthma.

The release of HIST, mainly from mast cells, may play a role in the pathogenesis of sepsis both in humans and in experimental animals [Brackett et al. 1990]. In animal models of sepsis and in ARDS patients, the plasma ET-1 concentration is significantly increased [Sanai et al. 1996, Mitaka et al. 1998]. The main mechanism responsible for the rise in ET-1 level is the upregulation of ET-1 synthesis, which occurs in part in the lungs.

ARDS is an endpoint syndrome of acute lung injury of diverse etiology, being a result of complex pathophysiological processes located mainly in the lung. The lung injury in ARDS is the result of an inflammatory process, which develops under the influence of vaso- and bronchoactive mediators produced by damaged or activated endothelial and epithelial cells, platelets and leukocytes that have been sequestered in the pulmonary microcirculation. ARDS is characterized by pulmonary hypertension, hypoxemia, increased microvascular permeability with interstitial and alveolar edema, increased airway resistance, decreased pulmonary compliance and a reduced functional residual capacity. The development of ARDS in most cases is associated with Gram-negative septicemia and septic shock. Thus, septically induced ARDS has frequently been studied in animal models, involving the infusion of live Gram-negative bacteria or bacterial lipopolysaccharide, i.e. endotoxin, into experimental animals. In these models, an acute pulmonary dysfunction occurred, which is the main feature of ARDS. Additionally, changes in the lung mechanics (decreased dynamic compliance and increased lung resistance resulting from active airway constriction) and pulmonary hypertension were consequences of the released mediators (i.e. HIST and ET-1, among others) [Forsgren and Modig 1986].

Bronchial asthma is a chronic inflammatory disease of the airways, characterized by airway narrowing and hyperresponsiveness to a variety of bronchoconstrictor stimuli. Airway inflammation can be defined as the presence of activated inflammatory cells (mast cells and eosinophils). These cells have the capacity to release potent bronchoconstricting mediators (e.g. HIST), which are responsible, at least in part, for airway narrowing, mucosal edema,

mucus secretion and structural changes, such as epithelial damage, and an altered smooth muscle function, including changes in the responsiveness or volume [Barnes 1998]. The HIST level in bronchoalveolar lavage has been shown to correlate with the parameters of disease activity in asthma [Casale et al. 1987]. The inadequate pulmonary removal of HIST may be an important factor in the mediation of pathophysiological responses, such as bronchial smooth muscle contraction, increased capillary permeability, mucus production and an increased airway permeability [Said 1982].

The airway epithelium has a protective function against noxious stimuli. The epithelial cells are able to release a variety of bronchoactive mediators, which modulate the function of the underlying smooth muscle cells. As ET-1 induces airway smooth muscle contraction and has a role in airway hyperresponsiveness, and as an upregulation of ET-1 expression occurs in the epithelial cells, ET-1 may have a potential pathophysiological role in bronchial asthma [Springall et al. 1991, Hay et al. 1993a, Barnes 1998].

Acute inflammation in the airways elicits changes in the airway mechanics, leading to bronchial hyperresponsiveness, which means an increased sensitivity and maximal response to bronchoconstrictor stimuli. In clinical practice and research, bronchoprovocation tests based on methacholine (MCh) or HIST inhalation is frequently used to assess the airway hyperresponsiveness.

Lung mechanics – Methodological overview

During breathing, the activity of the respiratory muscles results in changes in flow, pressure and volume in the lungs. Lung mechanics deals with the forces and resistances that determine the flow of air into and out of the lungs. The primary function of the lung is to meet the demand for O_2 in the tissues. The bronchial tree, extending from the trachea to the terminal respiratory units, conducts the air to the alveolar surface, where gas transfer occurs between the inspired air and the gas dissolved in the blood of the pulmonary capillaries. There are differences in structure and function between small (<2 mm in diameter) and large airways. The total cross-sectional area of small airways is greater than that of large airways. The flow is the same for all cross-sections, but the linear velocity of the gas (flow/cross-sectional area) is smaller in the small airways. Hence, small airways are characterized by laminar flow, whereas the flow in large airways is turbulent in consequence of the greater

linear velocities of the gas. In the lung periphery, the large cross-sectional areas result in lower overall resistance in the small airways. The airway resistance depends on the number, length, and cross-sectional area of the airways. Among these, the most variable component of the airway resistance is the cross-sectional area, which is determined, for a given anatomical structure, by the balance between two opposing forces: those tending to distend the walls (the retraction of the lungs by the negative pleural pressure) and those tending to contract the walls (airway smooth muscle and elastic elements). Neurohumoral mediators and the local changes in gas tension also influence the airway resistance and trigger compensation mechanisms to match ventilation/perfusion.

The airway smooth muscle is a major determinant of the airway caliber. The effect of muscle contraction depends on the location and the density of the muscle fibers. In the large airways, muscle contraction results in a high level of rigidity, as the muscle fibers oppose the tips of the cartilage, whereas in the medium and small bronchi both the caliber and the length of the bronchus are reduced during contraction. During contraction, the airway lumen decreases, to an extent depending on the shortening of the muscle fibers and the thickness of the airway wall. The extent of the shortening is determined by the tensile force generated in the muscle and by the magnitude of opposing forces (elastic elements in the airway wall and the surrounding parenchyma).

In the early days of respiratory research, the mechanical properties of the lung were characterized by resistive losses due to the changes in airway diameter, and the elasticity attributed to the lung parenchyma. Conventionally, to estimate the resistance and elastance of the respiratory system under physiological conditions requires the measurement of flow, volume and transrespiratory pressure. However, it was demonstrated that the parenchymal tissue is a viscoelastic material associated with energy losses [Bayliss and Robertson 1939], and it exhibits hysteretic behavior both *in vivo* [Ludwig et al. 1987, 1992] and *in vitro* [Fredberg et al. 1993], due to different structural materials (collagen-elastin-proteoglycan matrix and surfactant under control conditions, and contractile elements during constriction). The tissue constriction can be caused by constriction of the smooth muscle elements in the lung parenchyma, the tissue matrix and the alveolar ducts, which results in changes in the alveolar geometry and affects the rheological properties of the surfactant.

Thus, the total lung resistance (R_L) is the sum of the airway resistance (Raw), which is the resistance to flow in the airways, and the tissue resistance (Rti), which reflects the

dissipative energy losses of the lung parenchyma. Rti has been found to account for the major proportion of R_L under control conditions [Ludwig et al. 1987, Brusasco et al. 1989, Nagase et al. 1992, Ingenito et al. 1993, Nagase et al. 1997]. The tissue component has been reported to be responsible for 25-82% of R_L in dogs [Brusasco et al. 1989], 60% in rabbits [Nagase et al. 1992], 27-33% in guinea pigs [Ingenito et al. 1993] and 33% in rats [Nagase et al. 1997].

During constriction, bronchoactive agents exert significant effects on both Raw and Rti. The responses of Rti may even exceed those of Raw [Kariya et al. 1989, Hantos et al. 1992b, Romero et al. 1992, Ingenito et al. 1993, Pellegrino et al. 1993, Robatto et al. 1993, Nagase et al. 1994].

The development of the alveolar capsule technique [Fredberg et al. 1984, Brusasco et al. 1989, Hantos et al. 1992b] made it possible to partition R_L into airway and tissue components and to investigate the role of the lung parenchyma in R_L. The results achieved with this frequently used technique have confirmed the importance of the tissue resistance in both normal and constricted lungs. However, it is an invasive technique, which limits its applicability, and the alveolar pressure (P_A) can be measured only on small local areas of the lung. Since P_A exhibits a significant heterogeneity during constriction, the P_A values on which the partitioning of R_L into Raw and Rti is based may be highly accidental [Hantos et al. 1992b, Peták et al. 1993, Nagase et al. 1994].

Another means of separation of the airway and tissue components is the noninvasive assessment of respiratory mechanics by the forced oscillation technique. This was originally developed for human medicine [DuBois et al. 1956], but it has also been applied extensively to animals. Briefly, the respiratory mechanical impedance can be measured, with analysis of the responses (pressure and flow) of the respiratory system to externally generated, multiple-frequency signals. The respiratory impedance contains complex information on the resistive, elastic and inertial properties of the respiratory system [Peslin and Fredberg 1986], although at the usual oscillation frequencies (above 2-5 Hz) the drawing of inferences on the airway and tissue components is not straightforward.

The measurement of low-frequency pulmonary input impedance [Hantos et al. 1986] and the derived model parameters adequately characterize the mechanical properties of the airways and lung tissues in control states [Hantos et al. 1987, 1992a,b, Peták et al. 1993, Lutchen et al. 1994, 1996,] and during pulmonary constriction [Hantos et al. 1992b, Lutchen et al. 1994, 1996].

Histamine

In lung pharmacology, the early findings concerning the mechanical effects of HIST on isolated airway preparations demonstrated size- and species-dependent effects on the airways. Both bronchiolar and larger airway preparations from dog lungs responded with a marked contraction to HIST, with the same sensitivity [Persson and Ekman 1976].

It has been shown that bronchoactive agents (HIST, MCh, acetylcholine and serotonin) cause dose-related changes in the mechanical properties of the airways and parenchyma in whole lungs. The lung mechanical responses to these drugs are generally manifested as increases in R_L and simultaneous decreases in dynamic compliance [Colebatch et al. 1966, Hirshman et al. 1980, Mitzner et al. 1992].

HIST has been demonstrated to affect the mechanical properties of the airways and lung tissues by increasing Raw and Rti [Colebatch et al. 1966, Fredberg et al. 1985, Ludwig et al. 1987, 1989, 1991, Lauzon et al. 1992, Bates et al. 1993, 1994, Lutchen et al. 1994]. However, various data have been reported on the relative contributions of Raw and Rti to the increase in R_L. Lauzon et al. [1992] and Bates and Peslin [1993] found that smooth muscle contraction in the peripheral bronchi and blood vessels results in changes in the lung tissue mechanics and that the constriction in the central airways leads to the changes in Raw.

Additionally, Mitzner et al. [1992] suggested that, after administration of a bronchoconstrictor agent, the airway constriction passively distorts the lung parenchyma via the airway-parenchyma mechanical interdependence. They concluded that the intraparenchymal contractile cells were not involved in the changes in parenchymal mechanics.

In the present study, therefore, we examined the relationship between the airway and tissue responses to iv. HIST, and addressed the hypothesis that bronchoconstriction accounts for the greater part of the lung tissue response.

Methacholine

MCh is a synthetically available, chemical analog of acetylcholine, a parasympathomimetic agonist stimulating muscarinic receptors.

A number of studies have demonstrated that the mechanical effects of MCh depend on the route of delivery. In a canine model, R_L increased, while the static lung compliance

decreased during the iv. infusion of MCh [Breen et al. 1987]. Sato et al. [1993] found that the elevations in R_L and tissue elastance were accompanied by inhomogeneous ventilation of the lungs after MCh administration. In guinea pigs, significantly higher airway responses were accompanied by a significantly elevated Rti [Ingenito et al. 1993]. However, in rats, greater tissue responses than airway constrictor responses were observed after iv. MCh administration [Nagase et al. 1994]. Moreover, Salerno et al. [1995] demonstrated that the iv. infusion of MCh activated the peripheral contractile elements, leading to increases in Rti, dynamic elastance and hysteresivity (η). Lutchen et al. [1996] reported equal increases in Raw and tissue damping (G) (44-46%) during MCh infusion; however, the increase in tissue elastance (H) was much smaller.

Experiments with aerosolized MCh resulted in different data from those with iv. MCh [Kariya et al. 1989, Romero et al. 1992, Pellegrino et al. 1993, Robatto et al. 1993, Nagase et al. 1994]. Inhalation of MCh increased Raw and Rti to similar degrees in dogs [Robatto et al. 1993]. Romero et al. [1992] reported substantial changes in the intrinsic viscoelastic properties of the lung parenchyma in rabbits, without a change in Raw. In healthy and asthmatic human subjects, aerosolized MCh did not elicit tissue responses, but changes in the airways were observed [Kariya et al. 1989, Pellegrino et al. 1993].

The above-mentioned animal studies involved the use of an alveolar capsule technique to measure P_A, and the sampling of P_A may lead to false estimations of airway and tissue responses to a constrictor agent [Hantos et al. 1992b], especially in the case of an enhanced peripheral inhomogeneity due to MCh [Nagase et al. 1994].

Our goal was to study the effects of the iv. administration of MCh on the mechanical properties of the airway and tissue, with special regard to the relative contributions of the airways and parenchyma to the MCh-induced constriction. In the experiments, the low-frequency impedance (Z_L) was measured, and the airway and tissue responses were separated by fitting a model containing airway and parenchymal compartments to the Z_L data.

Endothelin

Cardiovascular system

The endothelins (ETs) are a family of three peptides (ET-1, ET-2 and ET-3), each consisting of 21 amino acids. All three isoforms have impressive biological effects. ET-1 was

originally isolated from a conditioned medium of cultured porcine aortic endothelial cells [Yanagisawa et al. 1988]. ET-1 is produced from preproendothelin-1, and proendothelin-1 or big endothelin-1 [Rubanyi and Polokoff 1994]. The circulating levels of ET-1 are very low, but its concentration at the endothelium/vascular smooth muscle interface is much higher than in the blood. ET-1 is released mostly abluminally, where it binds to the specific receptors on the vascular smooth muscle cells. ET-1 may, therefore, act locally, and should be regarded as a locally-acting paracrine hormone rather than as a circulating endocrine hormone. Though the circulating levels of ET-1 do not reflect the local production of this peptide, plasma ET-1 measurements may be useful and of diagnostic value in different disease states [Haynes and Webb 1993, Rubanyi and Polokoff 1994]. The synthesis and secretion of ET-1 is induced by different stimuli, such as epinephrine, thrombin, cytokines, hypoxia, ischemia and shear stress. Moreover, the production of ET-1 is regulated by inhibitory stimuli; endothelium-derived nitric oxide and prostacyclin may contribute to the overall hemodynamic effects of ET-1 [Vane and Botting 1991]. Under normal physiological conditions, the balance between the production of ET-1 and nitric oxide contributes to the maintenance of the basal vascular tone [Rubanyi and Polokoff 1994]. After iv. injection, ET-1 quickly disappears from the circulation because of the elimination by the lungs during the first passage [De Nucci et al. 1988].

Two types of ET receptors (ET_A and ET_B) have been cloned on the cells of mammalian species, and a putative ET_C receptor type has also been suggested [Sakurai et al. 1990, Lippton et al. 1993]. ET_A receptors are present on the smooth muscle cells of blood vessels, while ET_B receptors exist mainly on endothelial cells; however, they are also present on the smooth muscle cells of certain vascular beds. ET_A receptors preferentially bind ET-1, whereas ET_B receptors display an equal affinity for all isoforms of ET. The vasoconstrictor effects of ET-1 are mediated predominantly through the ET_A receptors, while activation of the ET_B receptors cause both vasoconstriction (ET_{B2}) and vasodilation (ET_{B1}) in certain blood vessels [Clozel et al. 1992, Rubanyi and Polokoff 1994]. The vasodilator action is probably linked to the increased production of nitric oxide and prostacyclin [De Nucci et al. 1988].

Lung

Besides the profound action in the cardiovascular system, the ETs play important roles in the regulation of the pulmonary function under physiological and pathological conditions.

Several studies on the synthesis of ET-1 in the tracheal and bronchial epithelial cells, type II pneumocytes and alveolar macrophages [Durham et al. 1993, Markewitz et al. 1995, Kobayashi et al. 1997] have revealed the importance of this peptide in the lung. ET-1 produces paracrine effects, as it is synthetized in the airway epithelium and elicits constriction in the adjacent smooth muscle cells. The peptide also causes smooth muscle cell proliferation, increased mucus secretion and activation of alveolar macrophages [Michael and Markewitz 1996].

Autoradiographic studies have identified specific binding sites for ET-1 in the airways of various animal species and of the human [Henry et al. 1990, Goldie et al. 1996]. In some species (human, guinea pig and rat), but not in mouse, there was a positive correlation between the airway responsiveness to ET-1 and the density of ET-1 binding sites [Henry et al. 1990]. Studies on the cellular locations demonstrated ET-1 binding sites in the alveolar capillary endothelial cells, bronchial smooth muscle cells, epithelial cells, alveolar type II fibroblasts and pneumocytes [Goldie et al. 1996]. The two receptor types coexist in the lung; however, there are interspecies differences in the proportions of the ET_A and ET_B receptors [Henry et al. 1990, Hay et al. 1993b, Goldie et al. 1996]. In the guinea pig lung, the ET_B receptors predominate (ET_B 80%; ET_A 12%) [Goldie et al. 1996]. Differences in the proportions of the two receptor types throughout the respiratory tract within a species have also been demonstrated: a higher density of ET_B receptors was found in the bronchi than in the trachea [Hay et al. 1993b].

Lung mechanical changes

On a molar basis, ET-1 is a less potent constrictor in the airways than in the vascular tissues. *In vitro* and *in vivo* studies have indicated ET-1-induced bronchoconstriction [Macquin-Mavier et al. 1989, Schumacher et al. 1990, Pons et al. 1991, White et al. 1991, Lueddeckens et al. 1993, Noguchi et al. 1993, Battistini et al. 1994, Nagase et al. 1995, Polakowski et al. 1996,], which is mediated by both receptor types in the guinea pig lung [Battistini et al. 1994, Nagase et al. 1995]. However, these studies inferred bronchoconstriction from the changes in the global lung mechanical parameters. For example, ET-1 elicited dose-dependent increases in the peak pulmonary inflation pressure (PIP) [Pons et al. 1991, Lueddeckens et al. 1993, Noguchi et al. 1993], or in RL, with a concurrent

decrease in the dynamic lung compliance following iv. injection into guinea pigs [Macquin-Mavier et al. 1989, Schumacher et al. 1990, White et al. 1991, Polakowski et al. 1996]. The measurements of these global lung mechanical parameters relate to the changes in the mechanical properties of both the airways and the parenchyma. The only study in which the airway and parenchymal responses to ET-1 were separated was reported by Nagase et al. [1995], who measured the local P_A through one or two capsules in guinea pigs. However, as noted above, separation based on measurements of P_A involves uncertainties because of the heterogeneity of P_A [Peták et al. 1993].

The aims of our studies were to investigate the dose-response characteristics of the airways and tissues to ET-1, and to establish how ET-1 alters the separate mechanical properties of the airways and the parenchyma in guinea pigs.

Selective and non-selective ET receptor antagonists are available which can facilitate a better understanding of the effects of the different ET receptor types in the biological responses and the mechanism of ET-1 action. BQ-610, a specific antagonist of the ET_A receptors [Ishikawa et al. 1992], is the linear derivative of the cyclic pentapeptide BQ-123. It is more potent than BQ-123 and more selective for ET_A than for ET_B receptors. ETR-P1/fl, another type of ET_A receptor antagonist [Baranyi et al. 1995], is a synthetic 15 amino acid, "antisense-homology box"-derived peptide with anti-ET_A receptor activity both *in vitro* and *in vivo*. IRL 1038 is a selective antagonist of the ET_B receptor [Urade et al. 1992]; it has much higher affinity for ET_B receptors than for ET_A receptors and inhibits the endothelium-dependent relaxation induced by ET-1 in the isolated rat aorta.

The aim of this study was to assess whether pretreatment with ET receptor antagonists (BQ-610, ETR-P1/fl and IRL 1038) influences the particular pulmonary responses to the administration of exogenous ET-1. Two ET-1 doses were chosen from those used in our previous study [Adamicza et al. 1999]: a smaller dose caused slight and homogeneous changes, and a higher dose elicited significant changes in the airway inertance, suggesting an inhomogeneous peripheral airway constriction.

In both studies involving an ET-1 challenge, a model-based evaluation of Z_L was used to determine the mechanical responses in the airways and lung tissues.

AIMS OF THE THESIS

The overall purpose of the research rescribed in this thesis was to investigate the lung mechanical responses to three bronchoactive agents (HIST, MCh and ET-1) that are of particular pathophysiological significance in respiratory research.

We examined the airway and lung tissue mechanical properties by using the low-frequency forced oscillation technique to measure Z_L. The airway and lung tissue responses were separated by fitting the Z_L data to a model containing the two compartments. This separation is based on the fact that the frequency-dependent characteristics of the airway and tissue components are different.

The concrete aims of the work reported here are as follows:

To examine the relationship between airway and tissue responses to iv. HIST, and to test the hypothesis that the bronchoconstriction accounts for the greater part of the lung tissue response in dogs.

To study the effects of the iv. administration of MCh on the mechanical properties of the airways and the parenchyma, and in particular the relative contributions of these compartments to the MCh-induced constriction in rats.

To investigate the dose-response characteristics of the airways and tissues following iv. ET-1 administration, and the mechanism whereby ET-1 alters the separate mechanical properties of the airways and the parenchyma in guinea pigs.

To reveal the role of the ET receptors in the mechanical responses of the pulmonary airways and tissues to iv. ET-1 in guinea pigs.

MATERIALS AND METHODS

Animal preparations

The studies were approved by the University Ethical Committee for the Protection of Animals in Scientific Research, and were performed in adherence to the NIH guidelines on the use of experimental animals.

Histamine challenge

Six mongrel dogs of either sex weighing 23.2 ± 2.6 kg were studied. The animals were anesthetized with pentobarbital sodium (30 mg/kg iv.), and a catheter was introduced into a femoral artery for monitoring of the systemic arterial blood pressure (BP). Tracheotomy was performed, and a metal tube (15-mm inner diameter) was inserted into the trachea. Mechanical ventilation was maintained with a Harvard respirator at frequencies of 12-15 breaths/min and a tidal volume of 20 ml/kg, with 5-cmH_2O positive end-expiratory pressure. Bilateral incisions were then made between the fifth and sixth ribs, and the sternum was split and widely retracted. The dogs were paralyzed with a dose of 0.1 mg/kg of pipecuronium bromide. Supplemental doses of pentobarbital sodium (10 mg/kg) and pipecuronium bromide (0.025 mg/kg) were given hourly to maintain a stable level of anesthesia and paralysis, respectively.

Methacholine challenge

Experiments were performed on five adult male Sprague-Dawley rats (330-450 g). The animals were anesthetized with pentobarbital sodium (30 mg/kg ip.) and placed in a supine position on a heating pad. A carotid artery and a jugular vein were cannulated for monitoring of the BP and for drug delivery, respectively. Tracheostomy was performed, and a 30-mm plastic cannula (2-mm inner diameter) was inserted into the trachea. Mechanical ventilation was maintained with a Harvard small-animal respirator, with a tidal volume of 3 ml and a frequency of 90/min. The end-expiratory pressure was set at 2.5 cmH_2O, the thorax was opened with a midline sternotomy, and the ribs were widely retracted. Paralysis was accomplished with pipecuronium bromide (0.2 mg/kg initial dose, supplemented every 20 min by 0.05 mg/kg). Additional anesthetic (10 mg/kg pentobarbital sodium) was given every 40 min.

Endothelin challenge without and with endothelin receptor antagonists

The experiments were carried out on guinea pigs (weighing 380-600 g) anesthetized with pentobarbital sodium (30 mg/kg ip.). The animals were placed on a heating pad to maintain the body temperature at 37 °C. The right carotid artery and left jugular vein were cannulated for the measurement of BP and drug administration, respectively. A plastic cannula (length=30 mm, internal diameter=2 mm) was inserted into the trachea and connected to a small-animal respirator (Harvard App. Inc., South Natick, MA, USA) delivering a tidal volume of 6 ml/kg at a frequency of 70/min. Following a midline sternotomy, the chest was widely opened and a positive end-expiratory pressure of 2.5 cmH_2O was applied. The guinea pigs were paralyzed with pipecuronium bromide (0.2 mg/kg). Additional doses of pentobarbital sodium (10 mg/kg) and pipecuronium bromide (0.05 mg/kg) were given as needed to maintain anesthesia and paralysis, respectively.

At the end of the experiments, the animals were killed with an overdose of pentobarbital sodium, and lung tissue samples were taken. The ratio of the wet weight to the dry weight of the lungs (WW/DW) was calculated to estimate the amount of pulmonary edema. The WW/DW values obtained in the main study population were compared with those calculated from another group of untreated normal guinea pigs (n=7). Moreover, lung tissue samples were fixed in 10% neutral-buffered formalin and paraffin embedded. Sections of each sampled tissue were stained with hematoxylin-eosin and periodic acid-Schiff. The alveoli were inspected for edema fluid, polymorphonuclear cells and macrophages, and the alveolar capillaries for congestion.

Impedance measurements and model fitting

Histamine challenge

Small-amplitude pseudorandom volume oscillations (in the frequency range 0.2-21.1 Hz) from a computer-controlled closed-box loudspeaker system were introduced into the trachea. The volume delivered was ~40 ml peak-to-peak at the airway opening; this corresponded to tracheal airflow values <220 ml/s peak-to-peak. Airway opening pressure was measured with a Validyne MP-45 transducer (Northridge, CA) via a side-tap of the tracheal tube, with reference to atmospheric pressure. The tracheal airflow was measured with a heated screen pneumotachograph and a Validyne MP-45 transducer. The controlling computer was also used for the processing of the measured signals. Z_L was computed from

the cross-spectra of the flow and pressure signals with the excitation signal, as described previously (Hantos et al. 1992b).

Challenges with methacholine or endothelin without and with endothelin receptor antagonists

The wave-tube method was applied to measure the mechanical impedance of the respiratory system [Van de Woestijne et al. 1981], with the modification for small animals [Hantos et al. 1987, Peták et al. 1997]. A loudspeaker-in-box system and the tracheal cannula were connected through a polyethylene wave tube (length=105 cm, inner diameter=2 mm) during apneic periods. The mechanical ventilation was interrupted at end-expiration and a forcing signal was introduced into the trachea. Before each measurement, the pressure in the box chamber was adjusted to 2.5 cmH_2O to keep the transpulmonary pressure unchanged during measurements. Lateral pressures were measured with ICS transducers (IC Sensors, Milpitas, CA) at the loudspeaker end (P_1) and the trachea cannula end (P_2). The computer-generated small-amplitude pseudorandom signal contained multiple frequency components between 0.5 and 21 Hz. The pressure signals were low-pass filtered and digitized by an analog-to-digital board of a computer. Fast Fourier transformation was used to calculate the pressure transfer function (P_1/P_2) spectra from the 6-s recordings by using 4-s time windows and 97% overlapping. Z_L was calculated as the load impedance of the wave tube by using the transmission line theory [Franken et al. 1981, Van de Woestijne et al. 1981]:

$$Z_L = Z_0 \sinh(\gamma L)/[(P_1/P_2) - \cosh(\gamma L)]$$

where Z_0 and γ are determined by the geometrical data and the material constants of the tube and the air.

Model fitting

A model containing an airway and a constant-phase tissue compartment was fitted to the Z_L data [Hantos et al. 1992a,b]; the term "constant phase" means that, in the tissue compartment, the real and imaginary parts decrease with the same power (α) of angular frequency (ω):

$$Z_L = Raw + j\omega Iaw + (G - jH) / \omega^{\alpha}$$

where j is the imaginary unit; the airways were characterized by the frequency-independent airway resistance (Raw) and inertance (Iaw); G and H are coefficients of tissue damping

(viscance) and elastance, respectively. Parenchymal hysteresivity (η) was calculated as the ratio of dissipated energy to the stored potential energy per unit deformation, $\eta=G/H$ [Fredberg and Stamenović 1989]. Impedance data points corrupted by the heart rate and its harmonics were omitted from the model fit.

Before the experiments, the resistance and inertance of the tracheal cannula were also measured, and were subtracted from the corresponding Raw and Iaw.

Experimental protocols and groups

Histamine challenge

Each oscillatory measurement was started after the animal was disconnected from the respirator at end-expiration, and lasted for 20 s. HIST (Sigma Chemical) was dissolved in normal saline and infused iv. in seven steps, beginning with a rate of 0.25 μg/kg/min (H1), with subsequent doubling up to 16 μg/kg/min (H2-H7). The measurements of Z_L were made in the control state, during infusions of HIST, and 60 min after the last infusion. Both in the control state and postinfusion, four measurements were made and the results from the repeated measurements were then ensemble-averaged. The time elapsed between every two consecutive measurements was 2 min. The measurements during the HIST infusion were made in an interval when the BP had reached a steady state. After each oscillatory measurement, the lung was hyperinflated by occluding the expiratory outlet of the respirator for one respiratory cycle to avoid atelectasis. Before the oscillations, the respirator was detached at end-expiration from the tracheal tube, which was connected to the loudspeaker chamber preinflated to 5 cmH_2O.

Methacholine challenge

MCh (methacholine chloride, Sigma Chemical) was dissolved in saline and infused at a rate of 1, 2, 4, 8 or 16 μg/kg/min. Before each infusion, the lungs were hyperinflated by occluding the expiratory outlet of the respirator for one cycle, i.e. producing two superimposed inspirations. Four successive measurements of Z_L, were made 1 min apart and the infusion was then started. The duration of each infusion was 10 min, and Z_L was measured 30 s after the onset of the infusion and every 1 min thereafter. The infusion was suspended for at least 15 min to allow the BP to recover before the next series of control and infusion measurements was begun.

Endothelin challenge

The measurements of ZL were made before and following every iv. bolus of ET-1, by doubling the doses from 0.125 μg/kg to 2 μg/kg. Prior to each dose, 3 to 6 measurements were made to establish the baselines. Before the control measurements, the lungs were hyperinflated by superimposing two inspirations to open the possible atelectatic areas. ZL was recorded 0.5, 1, 2, 4, 6 and 8 min after each ET-1 bolus. The ZL curves obtained in each of the control conditions were ensemble-averaged. The ZL data measured after each dose of ET-1 were fitted individually, and the parameter values obtained from the 0.5-min recording were selected to characterize the constrictor responses.

Endothelin challenge with endothelin receptor antagonists

Four measurements of ZL were made under the control conditions (before antagonist pretreatment and/or the administration of ET-1_1 (0.05 nmol/kg) and ET-1_2 (0.2 nmol/kg). Further ZL measurements were made at 0.5, 1, 2, 4 and 6 min after the administration of each of the successive ET-1 boluses. Prior to the control ZL measurements, the lungs were hyperinflated by occluding the expiratory line of the respirator for two consecutive cycles to prevent atelectasis. This maneuver was repeated at 7 min after each ET-1 dose. The control ZL spectra and those recorded after the administration of the ET receptor antagonist were averaged separately and then fitted, whereas the ZL data obtained following the administration of the two successive doses of ET-1 were fitted individually. The constrictor responses to each of the ET-1 doses were characterized by the parameter values obtained from ZL recorded at 0.5 min after administration.

After the surgical procedure, the animals were allowed to stabilize for 20 min. They were then randomized into one or other of the five following groups and given ET-1 iv. in two successive doses, the first ET-1 dose (ET-1_1) at zero time and the second dose (ET-1_2) 10 min thereafter (group *ET-1*, n=9), or 20 nmol/kg ET_A receptor antagonist BQ 610 (group *BQ-610_{20}*, n=8), or 80 nmol/kg ET_A receptor antagonist BQ-610 (group *BQ-610_{80}*, n=6), or 20 nmol/kg ET_B receptor antagonist IRL 1038 (group *IRL 1038*, n=9), or 20 nmol/kg ET_A receptor antagonist ETR-P1/fl (Kurabo Ltd., Osaka, Japan) (group *ETR-P1/fl*, n=5). The antagonist was administered 3 min prior to the first ET-1 dose (ET-1_1). ET-1, BQ-610 and IRL 1038 were products of Alexis Corporation (Läufelfingen, Switzerland).

Statistical analysis

Data are expressed as means ± SE. The statistical significance of changes in the lung mechanical parameters during HIST challenge was evaluated by using Wilcoxon's signed-rank test. The one-way analysis of variances with Dunnett's multiple comparison procedure was used to compare the parameters from the control with those obtained after MCh challenges. Friedman repeated measures analysis of variance on ranks with the Student-Newman-Keuls multiple comparison procedure, and Kruskal-Wallis one-way analysis of variance on ranks with the Dunn multiple comparison procedure were used for within-group and between-group comparisons, respectively, in the studies with ET challenges. A P value of <0.05 was considered statistically significant.

RESULTS

Histamine challenge

The dose-dependent changes in the pulmonary mechanical parameters tended to vary from dog to dog. The Raw values did not exhibit statistically significant differences from the control value except during the highest rate of infusion (Fig. 1). Iaw changed by less than ± 5% during the infusions and the only significant value was reached postinfusion.

Significant dose-related increases occurred in G and H at H3 and H4, respectively (Fig. 2). The mean values of G and H reached their maximum at H7; however, the changes in G (to 236% of the control value) were much higher than those in H (to 136% of the control value). η was significantly higher than the control value at H3, and increased to 169% of the control value at H7. All of the tissue parameters remained slightly but statistically significantly elevated after the last HIST infusion.

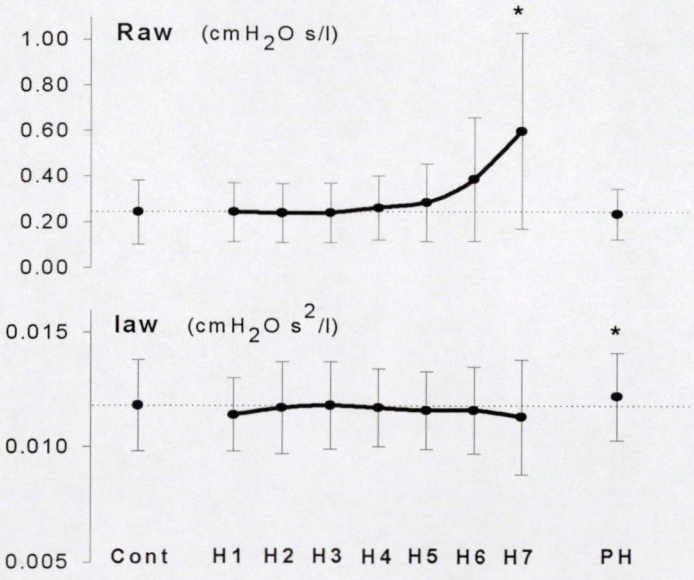

Figure 1. Airway parameters (*Raw* and *Iaw*) in the control state (*Cont*), during the infusion of HIST (*H1-H7*) and 60 min after infusion (*PH*). Dotted lines denote control (*Cont*) values.
* Significantly different from the control value.

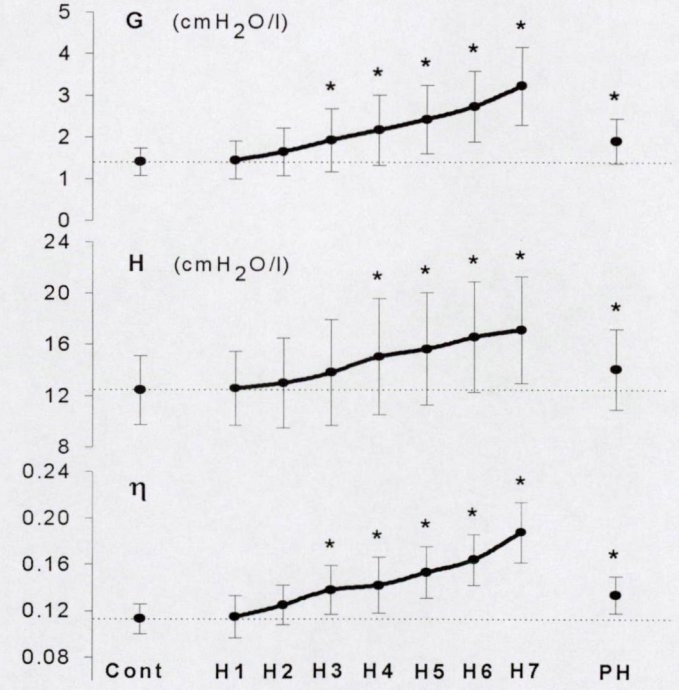

Figure 2. Lung tissue parameters (*G, H* and η) in the control state (*Cont*), during the infusion of HIST (*H1-H7*) and 60 min after infusion (*PH*). Dotted lines denote control (*Cont*) values. * Significantly different from the control value.

In Fig. 3, the G values normalized by the control data are plotted against the normalized H values for each dog. The higher increases in G than those in H are indicated by the upward deviations from the line of identity (η=constant). The postinfusion G and H values did not return to the control values (they positioned at the medium-rate infusions).

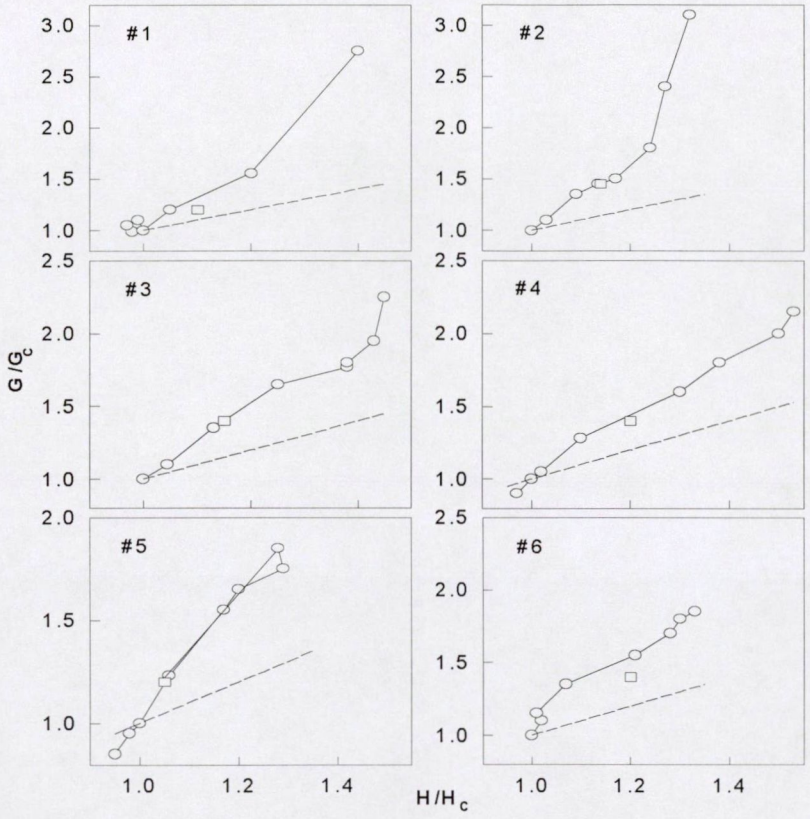

Figure 3. Relationship between tissue damping (G) and elastance (H) in the control state, during HIST infusion and 60 min after infusion (squares) in 6 dogs (#1-#6). Both parameters were normalized by control values (G_C and H_C). Dashed lines denote lines of identity corresponding to constant hysteresivity.

In Fig. 4, Raw is plotted against G for each dog, with both parameters normalized by their control values. The data demonstrate that elevations in G may occur before any change in Raw.

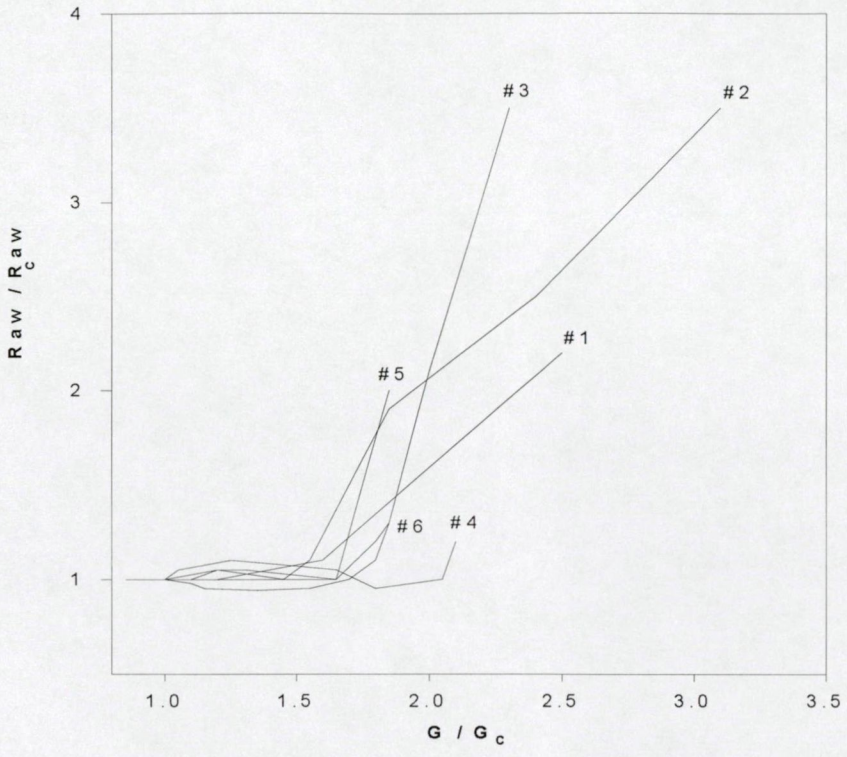

Figure 4. Relationship between airway resistance (*Raw*) and tissue damping (*G*), normalized by control values (Raw_C and G_C), in the control state and during HIST infusion, in 6 dogs (#1-#6).

Methacholine challenge

Figure 5 demonstrates the airway parameters obtained in the control state and at the different MCh-infusion rates. Raw increased in a dose-related manner, while Iaw remained unchanged, except during the highest infusion rate.

The G values increased significantly during the two higher infusion rates, whereas H remained at the control level (Fig. 6).

MCh induced similar elevations in Raw, G, and η, which reached 270 ± 90%, 340 ± 150% and 301 ± 102%, respectively, of their control values (Fig. 7). No residual effect of MCh was found, and every parameter returned to its baseline value between infusions (data not presented).

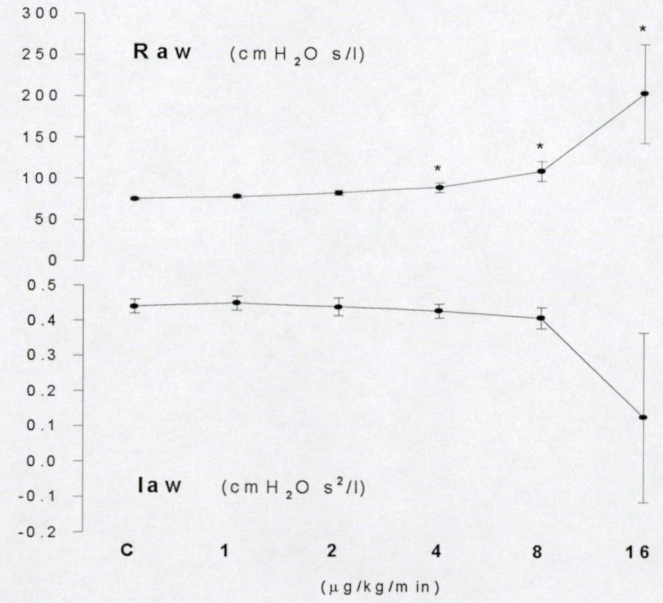

Figure 5. Airway parameters (*Raw* and *Iaw*) in the control state (*C*) and during MCh infusion.
* Significantly different from the control value.

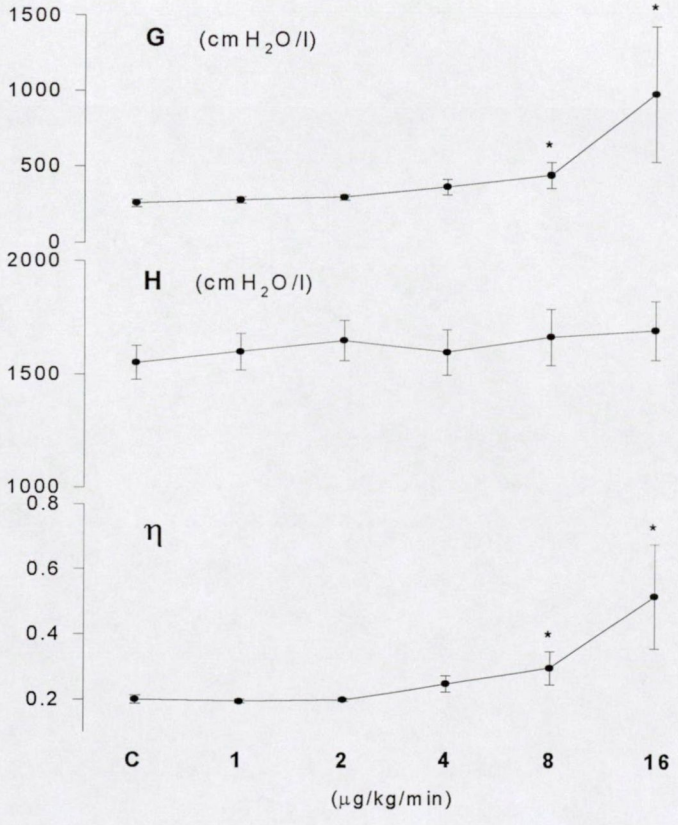

Figure 6. Lung tissue parameters (*G*, *H* and η) in the control state (*C*) and during MCh infusion.
* Significantly different from the control value.

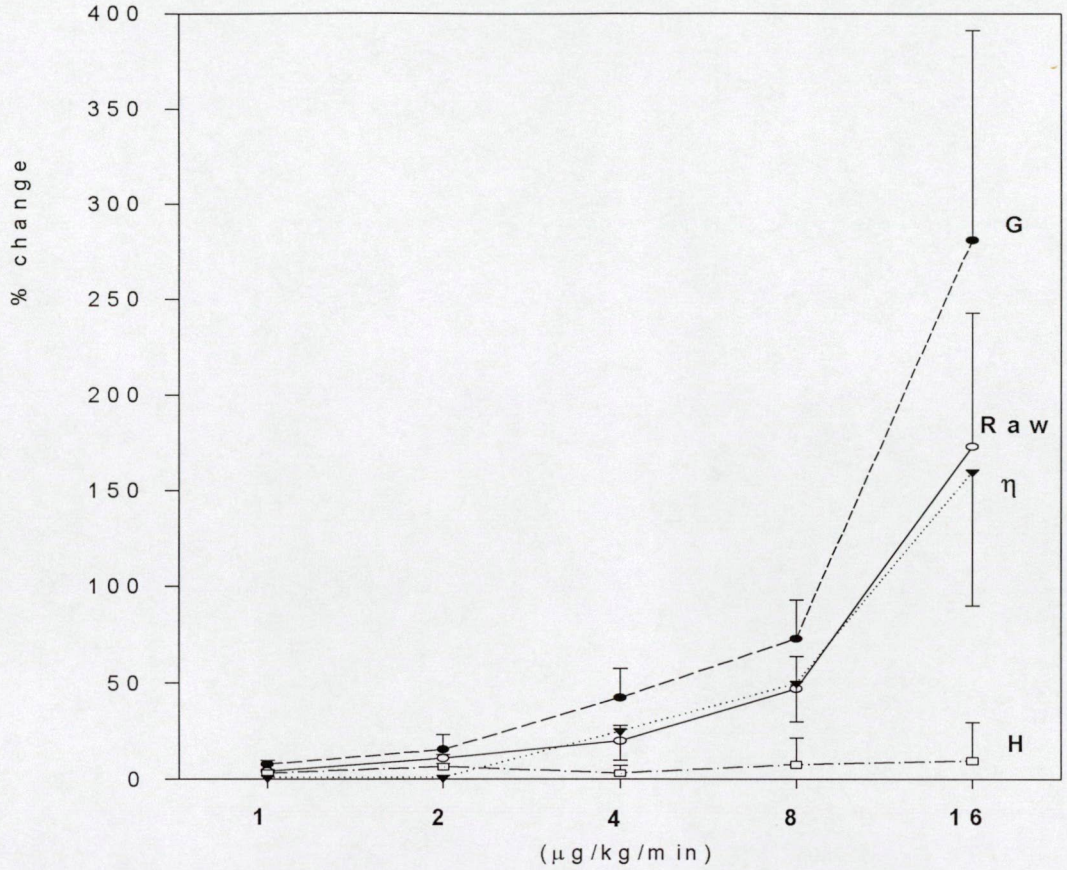

Figure 7. Percentage changes (normalized to the control values) in airway resistance (*Raw*), tissue damping (*G*), tissue elastance (*H*) and hysteresivity (η) during MCh infusion.

Endothelin challenge

Administration of increasing doses of ET-1 elicited statistically significant increases in BP (Fig. 8).

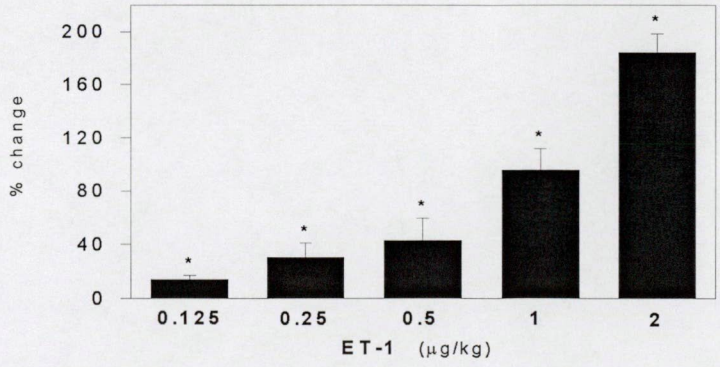

Figure 8. Effects of ET-1 on systemic blood pressure. % change = maximal value during ET-1 x 100/control value – 100. * $P < 0.05$.

Experimentally observed temporal changes in the airway and tissue parameters during successive administrations of ET-1 doses are presented in Fig. 9. The increasing doses of the constrictor agent induced monotonous elevations in Raw, G, H and η at low doses of ET-1, whereas no further increases in Raw and H were observed when the concentration of the ET-1 dose was increased from 1 to 2 μg/kg. All of the model parameters returned to the baseline following the low doses of ET-1; slight irreversible changes were seen only during severe constrictions. For all model parameters, peak responses occurred 30 s after the administration of ET-1 when the constriction was moderate (following the three or four lowest doses), while the parameter maxima were dissociated following the high doses of ET-1. Typically, G and η exhibited an instant peak increase after the injection of the bolus, whereas the peak response in H was slightly delayed. Raw occasionally followed the changing pattern of G (e.g. after 1 μg/kg in this example), or displayed a delayed increase (e.g. after 2 μg/kg). Despite this asynchrony, the parameter values observed 30 s after the ET-1 administrations adequately characterize the lung responses to most of the doses, although this sampling may lead to a slight underestimation of the increases in Raw and H during severe constriction.

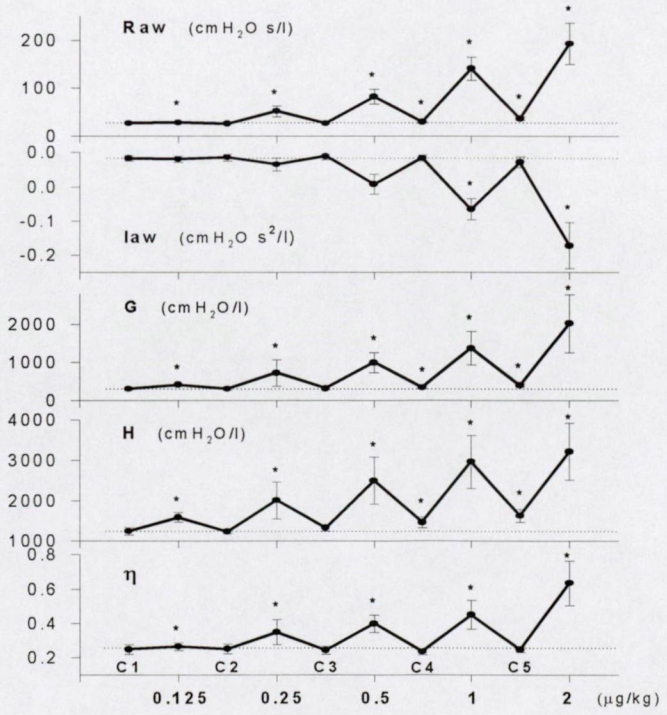

Figure 9. Airway (*Raw* and *Iaw*) and parenchymal (*G,H* and *η*) parameters in control conditions (*C1-C5*) and their peak responses to ET-1. Dotted line denotes baseline (*C1*) value. * Significant difference from the preceding control value.

Relative changes in the mechanical parameters in response to increasing doses of ET-1 are demonstrated in Fig. 10. The percentage increases for each parameter were calculated by normalizing the responses to the corresponding baseline values preceding each ET-1 dose. The marked increases in Raw and G occurred reasonably in parallel, while the elevations in H and η were smaller. The increases in tissue parameters were statistically significant following each ET-1 challenge, whereas Raw remained at the baseline after the lowest dose of ET-1, and only the last two doses of ET-1 induced marked and statistically significant decreases in Iaw.

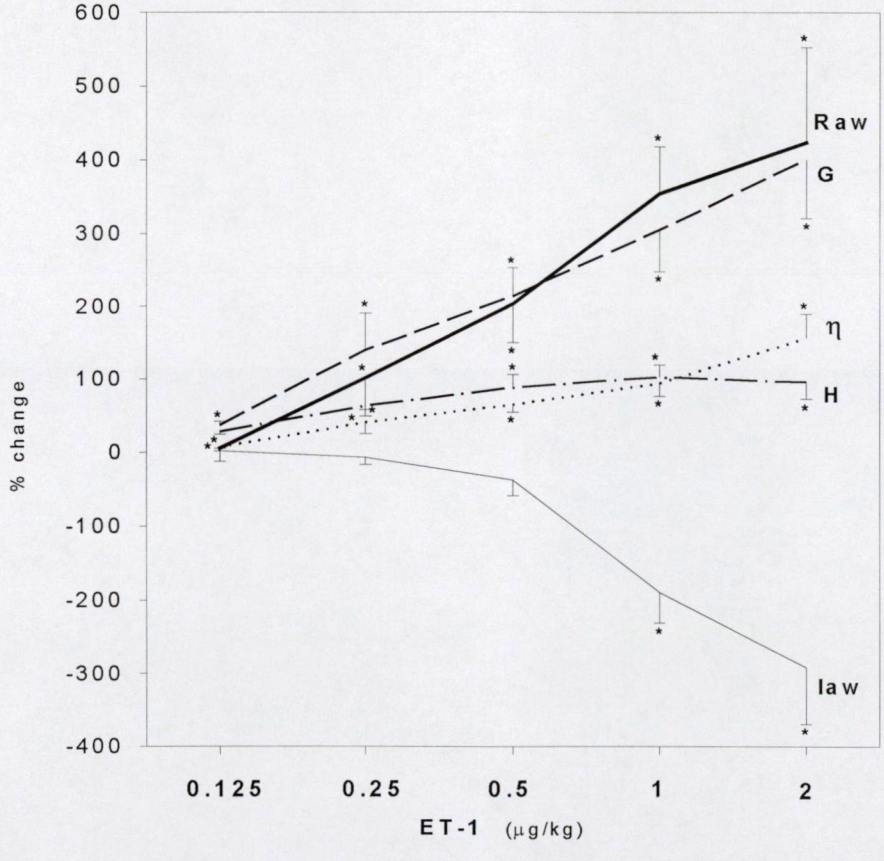

Figure 10. The effects of ET-1 on the values of the mechanical parameters. The responses in the parameters were normalized to the control values preceding each ET-1 dose. For definitions, see legend to Fig. 9. * $P < 0.05$.

The ratio WW/DW in the untreated group (4.7±0.13) was significantly lower than that obtained from the ET-1-challenged guinea pigs (5.2±0.14; $P<0.05$).

Endothelin challenge with endothelin receptor antagonists

In this study, the resting BP values were not significantly different between the groups. In group *ET-1*, ET-1 induced significant dose-dependent increases in BP (Fig. 11). The maximum values were reached within 1-2 min and BP then declined toward the baseline within 10 min. There were no significant differences in ET-1-induced BP changes between groups *BQ-610$_{20}$*, *IRL 1038* and *ET-1*, although ET-1$_2$ caused a somewhat smaller response in group *IRL 1038*. In groups *BQ-610$_{80}$* and *ETR-P1/fl*, the increases in BP were significantly less than that in group *ET-1*.

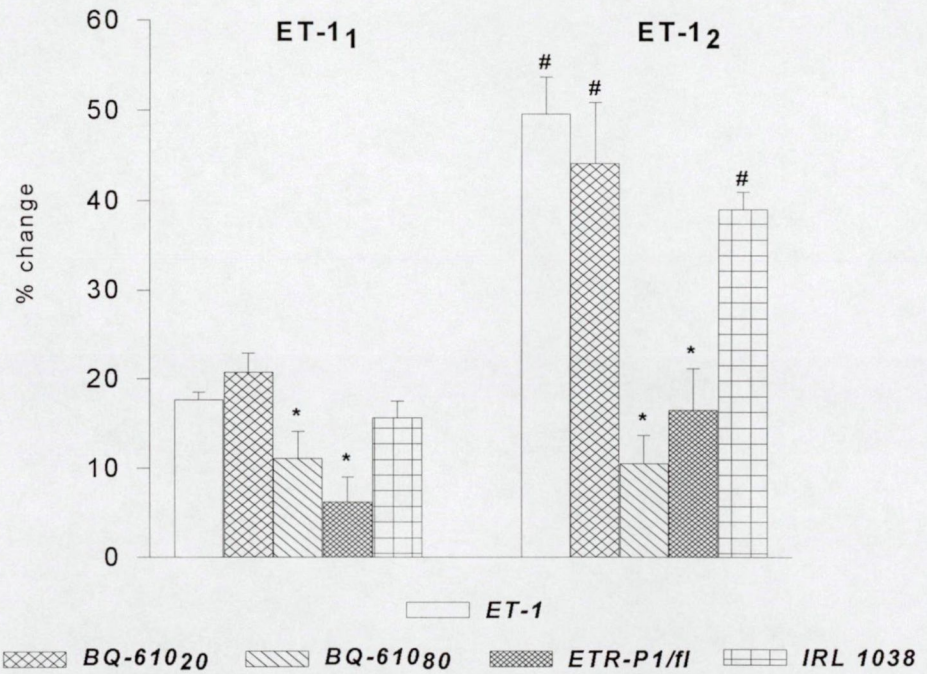

Figure 11. Peak responses in blood pressure induced by two ET-1 doses with and without pretreatment with ET receptor antagonists. The responses were normalized to the control values measured before ET-1 administration. * Significant difference from group *ET-1*. # Significant difference from ET-1$_1$ in groups *ET-1*, *BQ-610$_{20}$* and *IRL 1038*.

Figure 12 presents the characteristic profiles of the changes in the airway and parenchymal parameters after each of the two successive doses of ET-1, with and without pretreatment with the higher dose of BQ-610 or IRL 1038. There were only slight differences in baseline values between the individual groups; however, the control G value for group *BQ-610$_{20}$* was significantly different from that for group *ETR-P1/fl*. Both the first and the second

doses of ET-1 induced significant increases in Raw, G, H and η in group *ET-1*, and this dose-dependent pattern remained in all groups which received antagonists. The parameters reached their maxima within 30 s after the administration of either dose of ET-1, and the peak values then gradually declined toward the baseline. In group *BQ-610$_{20}$*, the elevations in the respiratory parameters were close to those observed in group *ET-1*. However, in groups *BQ-610$_{80}$*, *IRL 1038* and *ETR-P1/fl*, each of the doses of ET-1 evoked significantly smaller increases in Raw, G, H and η. The patterns of change in Iaw were inconsequential: in all groups but group *BQ-610$_{20}$*, no statistically significant changes in Iaw were observed after either ET-1 dose.

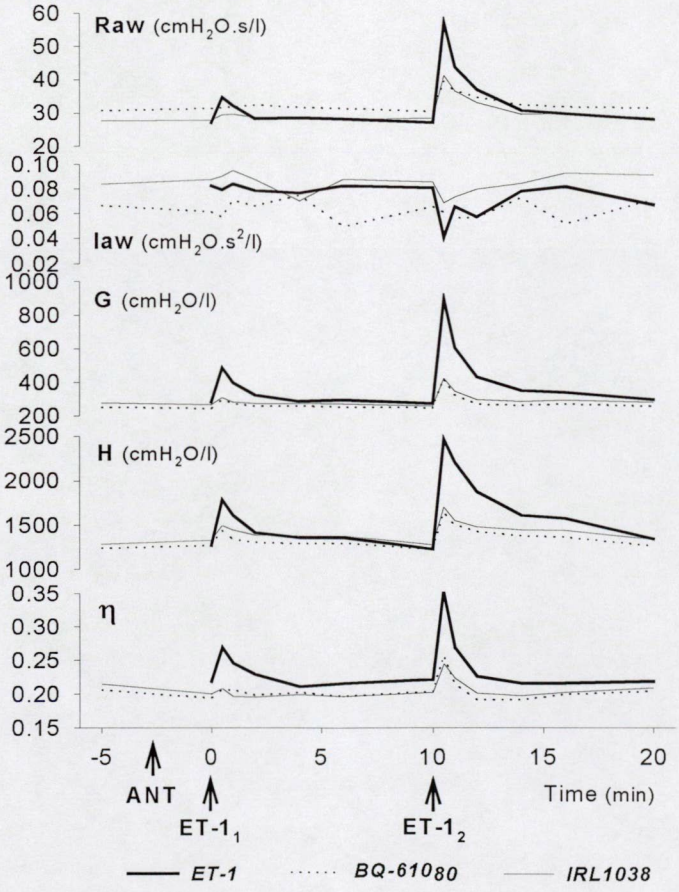

Figure 12. Mean airway (*Raw* and *Iaw*) and tissue (*G*, *H* and *η*) parameter profiles induced by two successive doses of ET-1 (ET-1_1 and ET-1_2) with and without pretreatment with one or other of two ET receptor antagonists. ANT: BQ-610_{80} or IRL 1038.

In group *ET-1*, the durations of significant changes in Raw and H were dose-dependent. Significant changes in η persisted for 1 min, and those in G for 10 min after each of the ET-1 doses (Table 1). The lower dose of BQ-610 significantly shortened the duration of changes in G, and increased those in η after ET-1_1. The responses in Raw, G and η were statistically significant only for short intervals in the cases of the higher dose of BQ-610, IRL 1038 and ETR-P1/fl.

Table 1. Mean durations (min) of significant changes induced in airway (Raw) and parenchymal parameters (G, H and η) by two successive ET-1 doses (ET-1_1 and ET-1_2), with and without pretreatment with an ET receptor antagonist.

Groups	Raw		G		H		η	
	ET-1_1	ET-1_2	ET-1_1	ET-1_2	ET-1_1	ET-1_2	ET-1_1	ET-1_2
ET-1	**1**	**6**	**10**	**10**	**6**	**10**	**1**	**1**
BQ-610$_{20}$	**6**	**4**	**1**	**6**	**6**	**10**	**4**	**1**
BQ-610$_{80}$	**0**	**0**	**0.5**	**1**	**1**	**6**	**0**	**0**
ETR-P1/fl	**0**	**2**	**0.5**	**1**	**10**	**6**	**0**	**0**
IRL 1038	**0**	**1**	**0.5**	**1**	**10**	**10**	**0**	**0**

Because of the high variations in the baseline data, the percentage changes in the airway (Raw) and parenchymal parameters (G, H and η) were calculated by normalizing the values measured at 30 s after each of the ET-1 doses to those obtained before ET-1 administration (Fig. 13). ET-1 induced dose-dependent increases in each of these parameters in group *ET-1*. In group *BQ-610$_{20}$*, the changes in most of the parameters did not differ significantly from the corresponding values in group *ET-1*; only the increase in H was significantly smaller after ET-1_1. The responses in Raw, G, H and η after each ET-1 dose were significantly smaller in groups *BQ-610$_{80}$*, *IRL 1038* and *ETR-P1/fl* as compared with those in group *ET-1*.

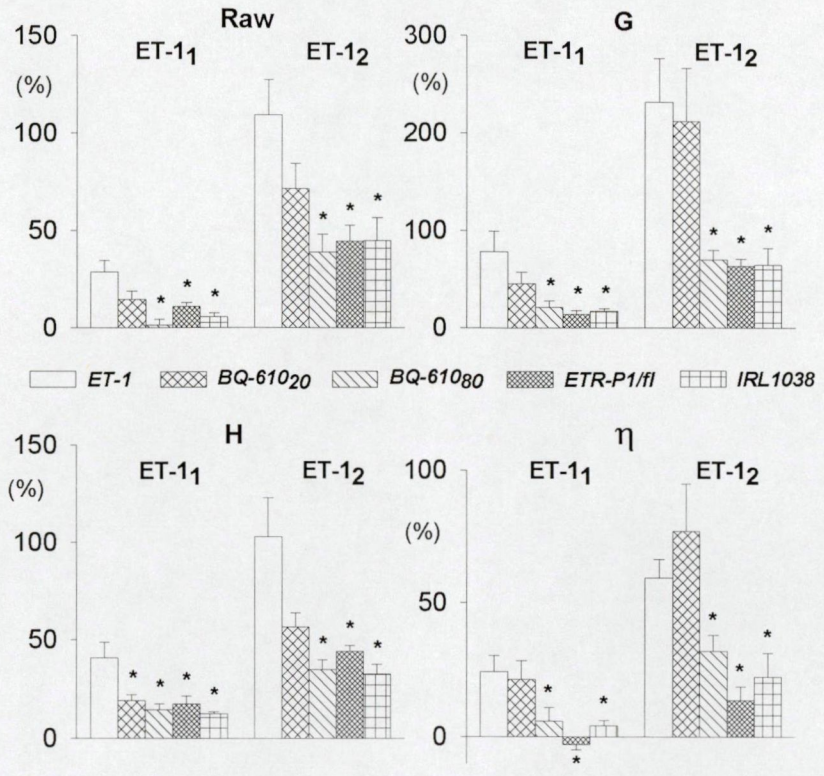

Figure 13. Changes induced in the parameters *Raw*, *G*, *H* and η by two successive ET-1 doses (ET-1_1 and ET-1_2) with and without pretreatment with an ET receptor antagonist. The parameters were measured at 30 s after each ET-1 dose and are normalized to the control values measured before ET-1 administration. * Significant difference from the value for group *ET-1*.

No significant differences were observed between the groups in the counts of polymorphonuclear cells and macrophages; moreover, no signs of alveolar edema were observed (data not shown). The absence of edema was supported by the results of gravimetric analysis of the tissue samples. For all the antagonist-treated groups, the ratio WW/DW did not differ significantly from that for group *ET-1* (*ET-1:* 5.11±0.1, *BQ-610*$_{20}$: 4.88±0.07, *BQ-610*$_{80}$: 5.63±0.2, *IRL 1038:* 5.06±0.1 and *ETR-P1/fl:* 5.19±0.1).

DISCUSSION

Histamine challenge

Our data suggest that an iv. HIST challenge can cause constriction in the lung periphery without any change in the overall Raw. This type of response to HIST demonstrates that the airway and tissue contractions could be dissociated. As the rates of HIST infusion elicit a

wide range of responses (from none or relatively small to large) in our study, we consider that this type of HIST challenge is appropriate for demonstration of the responsiveness of the airways and lung tissues.

HIST was earlier known only to induce mechanical changes in the lung due to the smooth muscle contraction of the airways, i.e. it reduced the airway diameter and increased the resistance. Subsequently, a number of authors described an increase in Rti [Colebatch et al. 1966, Ludwig et al. 1987, 1989] and changes in lung mechanical impedance [Fredberg et al. 1985, Ludwig et al. 1989] after a HIST challenge administered either iv. or as an aerosol. Several studies confirmed that HIST increases both Raw and Rti, although there were conflicting data on the relative contributions of the two components to the increase in RL [Fredberg et al. 1985, Ludwig et al. 1987, 1989, 1991, Lauzon et al. 1992, Bates et al. 1993, 1994, Lutchen et al. 1994]. Ludwig et al. [1987, 1989, 1991] found that the relative increases in Raw and tissue viscance were equal, the dose-response behavior of the two components of RL was similar, and the peripheral airways responded heterogeneously to HIST. However, in all of these studies, HIST was delivered by aerosol.

Mitzner et al. [1992] suggested that changes in lung tissue mechanics could occur without any contractile mechanisms in the parenchyma, for there is a mechanical interdependence between airway contraction and lung compliance during MCh infusion into the bronchial circulation in sheep. The contracted conducting airways lend stiffness to the surrounding parenchyma, and, according to the authors, this relationship may be a possible reason for the increased RL and a simultaneous decrease in dynamic compliance elicited by different bronchoactive agents. Lauzon et al. [1992] and Bates and Peslin [1993] suggested that the iv. HIST-induced smooth muscle contraction in the peripheral airways and pulmonary blood vessels was responsible for the changes in the tissue mechanical properties, whereas the changes in Raw were consequences of the constriction in the central airways.

Our findings are at variance with the hypothesis of Mitzner et al. [1992]: both G and H increased significantly, whereas there was no discernible change in Raw during the infusion of several doses of HIST: the only significant increase in Raw occurred during the highest infusion rate.

Intravenous HIST-induced bronchoconstriction is involved in ventilation inhomogeneity and this nonuniform constriction of the conducting airways causes significant, though virtual, changes in tissue parameters [Hantos et al. 1992b, Bates et al. 1994]. This may be possible

during severe constriction, such as during the highest infusion rate in the present study, and that by Lutchen et al. [1994]. Ludwig et al. [1991] demonstrated that a significant heterogeneity was evoked in the peripheral airways after HIST inhalation.

We observed significant increases in both tissue parameters during HIST infusion. Moreover, higher increases were found in G than in H. This process may be the result of peripheral inhomogeneity, as mentioned above, or of the intrinsic properties of the tissue, as demonstrated by Fredberg et al. [1993] in guinea pig parenchymal strips. The lung tissue has separate contractile elements with the ability to respond to bronchoactive agents, so that the primary response is a change in elastance; however, changes in the degree of coupling (η) between dissipative and elastic processes also occurred [Ludwig et al. 1989, 1991, 1992, Fredberg et al. 1993]. In our study, both H and η increased, as found previously during a HIST challenge [Loring et al. 1981, Lauzon et al. 1992, Bates et al. 1994, Lutchen et al. 1994], although, interestingly, no change in H occurred along the η=constant line (Fig. 3), demonstrating instantaneous, and possibly primary alterations in the intrinsic η.

The effect of HIST on the bronchial smooth muscle tone is complex; it involves both a direct contraction by stimulating the H_1-receptors in the airway smooth muscle [Dixon et al. 1979a, b], and an indirect one by stimulating afferent vagal fibers through rapidly adapting receptors in the airways [Vidruk et al. 1977]. H_2-receptor activity could not be demonstrated in the canine airways [Bradley and Russell 1983].

Our results reveal that constrictor agents may cause significant mechanical changes mainly in the lung tissue after HIST challenge, preceding any change in Raw. As concerns the interpretation of the findings, it must be taken into account that catecholamines released by the systemic administration of HIST can modify the mechanical changes [Staszewska-Barczak 1965, Colebatch and Engel 1974].

Methacholine challenge

This study documents a significant effect of iv. MCh on the airway caliber. As regards the airway parameters, the iv. delivery of MCh induced a threefold increase in Raw and no change in Iaw. As for the tissue parameters, an elevation in G similar to that in Raw occurred,

while no increases in H were found. The differences in behavior of the tissue parameters resulted in a significant increase in η.

Intravenous MCh significantly changed the airway caliber in a dose-dependent manner. It is known that an overwhelming contribution to Iaw is made by the inertance of the central airways. As Iaw remained at the baseline level or decreased slightly (except during the highest dose), we suggest that iv. MCh induced bronchoconstriction in the peripheral airways.

We observed a significant (fourfold) increase in G and no change in H during an iv. MCh infusion. These changes are only in partial agreement with those of Lutchen et al. [1996], who found, besides the significant increase in G, a statistically significant elevation in H. They suggested that during severe constriction the enhanced airway inhomogeneity can lead to an artifactual increase in G. Accordingly, there may be two possibilities for the changes in G. First, the MCh-induced increase in G can be a consequence of a changed intrinsic tissue damping, and second, it can be a result of the peripheral ventilation inhomogeneity. We suggest the latter phenomenon, i.e. we attribute the increase in G is attributed to inhomogeneous airway constriction during iv. MCh infusion. However, the possibility that the altered η also contributes to the elevation in G ($G = \eta \cdot H$) cannot be ruled out.

In a previous study that also involved Sprague-Dawley rats, significant airway responses were associated with elevated tissue responses; both Rti and dynamic elastance significantly increased [Nagase et al. 1994]. Others, using Brown Norway rats or guinea pigs, found large tissue responses [Ingenito et al. 1993, Salerno et al. 1995]. The reason for the difference between the reported changes in dynamic elastance and our H responses may be that dynamic elastance values are calculated at respiratory frequencies, whereas H is a frequency-independent elastance coefficient. As suggested by Lutchen et al. [1996], the peripheral airway inhomogeneities increase not only G, but also the positive frequency dependence of the effective elastance, whereas the intrinsic elastic and resistive properties of the tissues do not change.

MCh produces smooth muscle contractions by stimulating the muscarinic receptors in the airways [Barnes 1990]. Since the presence and distribution of the muscarinic receptor subtypes is species-dependent, the differences in the MCh responsiveness of the airways and the parenchyma are explained by this mechanism. The M3 receptors are located predominantly on the airway smooth muscle, and this receptor subtype may be responsible for

the airway constriction elicited by iv. MCh. M1 receptors have been detected in the human and rabbit peripheral lung, mainly on the alveolar walls, which have been reported to be involved in the parenchymal response, and can be reached more easily by aerosolized MCh. These interspecies differences must also be considered when the results on one species are compared with those on another.

There are quantitative differences in airway smooth muscle between the various species, and this may be an important cause of the variability in the responsiveness to bronchoactive agents [Martin et al. 1992]. Rats have the lowest sensitivity and the smallest maximal responses to MCh, while the larger quantity of airway smooth muscle in guinea pigs is associated with a larger maximal response to MCh.

Besides the differences in muscarinic receptor density and the quantity of airway smooth muscle, the effects of aging should also be considered. Nagase et al. [1994] found that increasing age decreased dynamic elastance after MCh inhalation in rats.

In summary, our results demonstrated that MCh induces changes in both the airways and the lung tissue, possibly via the stimulation of different receptors, with the airway constriction predominating.

Endothelin challenge

The airway responses to ET-1 were associated with significant changes in the mechanical properties of the lung parenchyma. Increasing concentrations of ET-1 induced progressive increases in Raw and monotonous increases in G, while H showed a plateau response at the three highest doses. In contrast, Iaw remained at the baseline level when the constrictions were mild, but exhibited significant decreases during severe constrictor responses. Although the changes in all mechanical parameters were fully reversible after the two lowest ET-1 doses, the higher doses caused irreversible parenchymal constrictions, which were associated with statistically significant residual increases in Raw. There were no changes in the baseline levels of Iaw or η.

The dose-dependent increases in Raw were associated with no increases in Iaw at low ET-1 doses. Since Iaw is thought to be a characteristic parameter of the central airways, this

changing pattern suggests a dominance of the periphery in the development of an ET-1-induced airway constriction.

At high ET-1 doses, Raw increased further, while significant decreases occurred in Iaw, even negative values being attained in most of the animals during severe constriction. This controversial changing pattern in the airway parameters can be explained as follows. In our study, Raw and Iaw characterize the mechanical properties of the overall bronchial tree. While both parameters are proportional to the overall length of the airways, Iaw and Raw are inversely related to the first and to the second power, respectively, of the overall bronchial cross-sectional area. Therefore, assuming no change in the overall airway length, we would expect both Raw and Iaw to increase with a generalized airway constriction. Although, in theory, Raw can increase while Iaw decreases if the airway constriction is associated with significant airway shortening, the unrealistic negative Iaw parameters obtained at high doses of ET-1 cannot be explained on that basis. Therefore, the opposite changes in Raw and Iaw during constriction can most probably be attributed to the failure of the model during a severe spatial inhomogeneity of the pulmonary constriction. By using a distributed periphery lung model to simulate ZL data in control and constricted conditions, Hantos et al. [1992b] demonstrated that, in the presence of an inhomogeneous peripheral constriction, the model-predicted Iaw systematically underestimates the real Iaw. Therefore, the opposite changes in Raw and Iaw at high ET-1 doses very probably indicate an inhomogeneous peripheral airway constriction.

We observed significant increases in both parenchymal parameters during iv. administration of ET-1. Since the increases in G exceeded those in H, significant elevations were obtained in η. In parenchymal strips obtained from guinea pig lungs, different constrictor agents induced greater increases in Rti than in the elastance [Fredberg et al. 1993]. Those literature data suggested that parenchymal constrictions were always associated with significant increases in the intrinsic η. Accordingly, ET-1 changed the coupling between the resistive and elastic properties at the level of the parenchyma, i.e. ET-1 may induce significant elevations in the intrinsic η.

Inhomogeneous peripheral airway constriction has been shown to increase the frequency dependence of RL [Hantos et al. 1992b, Lutchen et al. 1996, Peták et al. 1997] and create a significant artifactual component in G due to interregional peripheral ventilation [Lutchen et al. 1996]. In our study, as H increased significantly in response to ET-1, we

suggest that real intrinsic parenchymal constrictions occurred. The finding that, at the highest ET-1 dose, there was a significant elevation in G without any further increase in H suggests the role of enhanced peripheral inhomogeneity. Although the excessive increase in G may also be explained on the basis of a purely tissue reaction, i.e. an elevated η, the fact that this change is accompanied by a fall in Iaw supports the inhomogeneity as the underlying mechanism.

Following low ET-1 doses, each parameter returned to the baseline level; however, after severe constrictions, Raw, G and H remained above the baseline. A possible explanation for our findings may be that the lung remained constricted after the ET-1-induced responses. However, in this case, the parameter η would also be expected to remain above the baseline level [Fredberg et al. 1993]. A further possibility is that the residual changes can be attributed to lung volume loss. In our experiments, we performed hyperinflations to eliminate atelectatic regions, and therefore the lung volume loss is probably a result of pulmonary edema [Grossman et al. 1980], as supported by our finding that the ratios WW/DW were significantly higher after ET-1. Increases have likewise been observed in the vascular permeability after high doses of ET-1 [Filep et al. 1995].

The bronchoconstrictor effects of iv. ET-1 were concluded from the increase in RL and the decrease in dynamic lung compliance [Macquin-Mavier et al. 1989, Schumacher et al. 1990, White et al. 1991, Polakowski et al. 1996] or the increase in PIP [Pons et al. 1991, Lueddeckens et al. 1993, Noguchi et al. 1993]. However, the methods used in these previous studies do not allow a detailed investigation of the effects of ET-1 on the airway and lung tissue mechanics. It is known that PIP reflects the overall pressure losses in the lungs, including those of elastic origin, and RL has a flow resistive airway (Raw) and a viscoelastic parenchymal (Rti) component. Only Nagase et al. [1995] separated the ET-1-induced lung responses into airway and parenchymal components in guinea pigs and in mice [Nagase et al. 1996] by measuring the local PA. It has been pointed out that the sampling of one or two alveolar regions in a highly inhomogeneously constricted lung makes the capsule-based partitioning highly incidental [Hantos et al. 1992b, 1995, Peták et al. 1993, Lutchen et al. 1996, Peták et al. 1997]. In our study, the mechanical properties of the airways and the parenchyma were separated on the basis of a global lung mechanical model.

It is generally accepted that the lung parenchyma contributes to the constrictor response of the lungs. *In vitro* studies on parenchymal strips indicated that alterations occurred at the

parenchymal level, although the increases in the tissue mechanics were much smaller than those obtained for the whole lung [Fredberg et al. 1993]. Alternatively, according to Mitzner et al. [1992], airway constriction may lead to parenchymal responses via the mechanical interdependence between the airways and the lung tissues. In the present study, we found different time courses in the responses of the mechanical parameters. Following a high dose of iv.-administered ET-1, the peak responses were manifested first in G and η, while the maximal increases in Raw and H were somewhat delayed. Since several studies document that the airway and parenchymal constrictions are dissociated [Bates et al. 1994, Lutchen et al. 1994, Hantos et al. 1995, Suki et al. 1997], we may conclude that ET-1 induces lung tissue constriction by acting on the contractile elements in the parenchyma rather than as a result of airway contractions.

In summary, we have demonstrated that increasing doses of ET-1 affect the mechanical properties of the airways and the parenchyma in guinea pigs in a dose-related manner. The dose-response curves for the airway and parenchymal mechanical parameters suggest the dominance of the peripheral airways in the development of the ET-1-induced constriction. Besides a significant inhomogeneous constriction of the peripheral airways, real intrinsic increases occurred in the mechanical properties of the parenchyma. Our results further indicate that ET-1 has a marked constrictor effect not only on the vascular and bronchial smooth muscles but also on the lung parenchyma.

Endothelin challenge with endothelin receptor antagonists

This study confirms that the administration of an ET_A or ET_B receptor antagonist alone does not change the basal values of the lung mechanical parameters, which suggests that endogenous ET-1 does not play a significant role in the regulation of the resting bronchomotor tone in guinea pigs. However, each of the iv. doses of ET-1 significantly increased Raw, G, H and η. Pretreatment with the lower dose of BQ-610 significantly decreased the changes only in H after ET-1_1, whereas the higher dose of the antagonist also reduced those in Raw, G and η. Pretreatment with IRL 1038 or ETR-P1/f1 reduced the responses to ET-1 in both the airways and the parenchyma. Thus, it is concluded that ET-1

induces mechanical responses via the activation of both ET_A and ET_B receptors in the airways and the parenchyma in the guinea pig lungs.

In the present study, we used two ET_A receptor antagonists (BQ-610 and ETR-P1/fl) and a selective ET_B receptor antagonist (IRL 1038) to investigate the roles of the ET receptors in the mechanical properties of the airways and the parenchyma. BQ-610 is a highly selective ET_A receptor antagonist, being an approximately 30 000 times more effective inhibitor of ET_A than of ET_B receptors *in vitro* [Ishikawa et al. 1992], and it effectively antagonizes the *in vivo* effects of ET-1 on the ET_A receptors [Boros et al. 1998, Szalay et al. 1998]. ETR-P1/fl is an "antisense-homology box"-derived peptide, which exhibits anti-ET_A receptor activity in the cardiovascular system both *in vitro* [Baranyi et al. 1995] and *in vivo* [Boros et al. 1998, Szalay et al. 1998]. In addition, it improves the symptoms and hemodynamic parameters in endotoxin shock [Baranyi et al. 1998] and in hypodynamic septic rats [Szalay et al. 1998], and inhibits the endothelial cell-leukocyte interactions in response to ET-1 in the intestinal microcirculation in rats [Boros et al. 1998]. IRL 1038 binds specifically to the ET_B receptors in various mammalian tissues, including the guinea pig lungs [Urade et al. 1992].

In the literature, there are no data that are comparable with our recent [Adamicza et al. 1999] and present findings. Only Nagase et al. [1995] reported parenchymal changes after iv. administered ET-1 in guinea pigs *in vivo*.

In the present study, similarly as in our previous investigation [Adamicza et al. 1999], the two successive doses of ET-1 each caused significant dose-related changes in the mechanical properties of the airways and the parenchyma. Raw was increased significantly, without any significant change in Iaw. Since Iaw is dominated by the inertance in the central airways, we conclude that ET-1 induces airway constriction in the periphery of the lung.

Autoradiographic studies have demonstrated that ET_A and ET_B receptors coexist in the guinea pig lung, with an ET_A:ET_B receptor ratio of 1:4 [Goldie et al. 1996]. Furthermore, Hay et al. [1993b] described regional differences in the distributions of the two ET receptor types in the airways. The primary bronchi contain more ET_B receptors, whereas the ET_A receptors predominate in the trachea. The relative contribution of the ET_B receptors to bronchoconstriction therefore increases in the distal direction along the respiratory tract [Hay et al. 1993b]. Pharmacological investigations on isolated guinea pig bronchi provided evidence that ET-1 causes bronchoconstriction largely via ET_B receptor stimulation [Hay 1992, Battistini et al. 1994]. Nagase et al. [1995] found that both ET_A and ET_B receptor

antagonists effectively reduce the ET-1-induced increases in airway resistance *in vivo*. In our examinations, IRL 1038, ETR-P1/fl and the higher dose of BQ-610 significantly decreased the elevation in Raw induced by each of the ET-1 doses. Our data therefore indicate the role of both receptors in the ET-1-induced airway responses.

We observed significant increases in the parenchymal parameters G, H and η, which indicates changes in the mechanical properties of the parenchyma after ET-1. Pretreatment with IRL 1038, ETR-P1/fl or the higher dose of BQ-610 significantly diminished the responses in H after each of the ET-1 doses. In parenchymal strips, Battistini et al. [1994] found that the ET_A receptor antagonist BQ-123 inhibited the ET-1-induced contraction. Nagase et al. [1995] reported that BQ-123 did not alter the responses in Rti and lung elastance to ET-1; however, the ET_B receptor antagonist BQ-788 significantly decreased the responses in both tissue parameters. In our experiments, pretreatment with both doses of BQ-610, IRL 1038 or ETR-P1/fl significantly reduced the changes in H after ET-1. These findings emphasized the roles of both types of ET receptors in the mediation of ET-1-induced constriction in the parenchyma.

In the guinea pig lungs, it has been demonstrated that the ET_A receptor antagonist BQ-123 decreases the bronchoconstrictor responses to ET-1 in a dose-dependent manner [Noguchi et al. 1993, Battistini et al. 1994]. In our experiments, pretreatment with BQ-610 in a dose of 20 nmol/kg had no significant effect on the ET-1-induced airway and parenchymal responses. However, pretreatment with the higher dose significantly decreased the changes in the airway and parenchymal parameters. The dose-dependent effect of the ET_A receptor antagonist BQ-610 on the ET-1-induced pulmonary constriction provides additional evidence in favor of the hypothesis of other authors [Noguchi et al. 1993, Battistini et al. 1994, Hay and Luttmann 1997] that there may be an ET_A receptor antagonist-binding ET_B receptor subtype in the guinea pig lung.

Our study provides the first demonstration of the effects of the ET_A receptor antagonist ETR-P1/fl on the ET-1-induced mechanical changes in the lung. In the presence of ETR-P1/fl, significantly smaller increases in Raw, G, H and η occurred after each dose of ET-1 than those observed in untreated animals. Moreover, the peptide decreased the durations of the significant changes in Raw, G and η after each ET-1 dose. Thus, ETR-P1/fl affected the pulmonary mechanics in the same dose as did IRL 1038, but not BQ-610. Therefore, it is reasonable to suggest that ETR-P1/fl is not selective for ET_A receptors in the guinea pig lung.

These findings, similarly to those of Szalay et al. [1998], indicate that ETR-P1/fl may antagonize not only the ET_A, but also the ET_B receptors. Another explanation may be that ETR-P1/fl removes circulating ET-1, as was observed by Baranyi et al. [1998] in dogs.

ET-1 is known to be a proinflammatory mediator, as it induces intravascular leukocyte sequestration, and enhances albumin extravasation and edema formation in the guinea pig lung [Filep et al. 1995]. The ET-1-induced edema is mediated by the ET_A receptors in the trachea and the bronchi, but not in the parenchyma. In the present study, doses of ET-1 lower than those used by Filep et al. [1995] did not evoke lung edema, as evidenced by gravimetric and histological examinations. In agreement with our results, Macquin-Mavier et al. [1989] did not observe lung edema in ET-1-treated guinea pigs.

It has been found that various mediators can also contribute to the ET-1-induced mechanical changes in the guinea pig lung. The bronchoconstriction elicited by ET-1 could be mediated by thromboxane A_2 released via ET receptor activation [De Nucci et al. 1988, Pons et al. 1991, Lewis et al. 1998]. ET-1 can release the bronchodilator prostacyclin [De Nucci et al. 1988] and nitric oxide [Lewis et al. 1998]. Therefore, we suggest that the direct bronchoconstrictor effect of ET-1 can be either potentiated or counteracted by secondary mediators.

In summary, we have characterized the pulmonary mechanical responses to the administration of ET-1, both with and without pretreatment with ET_A and ET_B receptor antagonists, with a method appropriate for the separate quantification of the airway and parenchymal components. The results reveal that the activation of both ET_A and ET_B receptor subtypes is involved in the mediation of the mechanical changes in response to ET-1 in the airways and the parenchyma. Since selective ET_A receptor antagonist treatment suppressed the changes in the mechanical responses in a dose-related manner, we suggest the existence of a heterogeneous ET_B receptor population in the guinea pig lung.

CONCLUSIONS

The major findings of the present work are as follows.

After iv. administration, the effects of histamine, methacholine, and endothelin-1 on the lung mechanical properties can be characterized by a dose-related constriction via different sites of action.

In dogs, the responses of the airways and tissues to histamine were dissociated; histamine caused constriction in the lung periphery without changes in the bronchomotor tone.

In rats, methacholine induced predominantly airway constriction, although an increase in the viscous resistance of the lung tissue also occurred as a result of the increased ventilation inhomogeneity.

In guinea pigs, endothelin-1 elicited constriction in both the airways and the lung parenchyma; however, the effect in the peripheral airways appeared to predominate.

In guinea pigs, both ET_A and ET_B receptor subtypes were involved in the mediation of the mechanical changes in the airways and lung tissue. The fact that a selective ET_A receptor antagonist decreased the mechanical responses of the lung in a dose-related manner, supports the hypothesis that a heterogeneous ET_B receptor population exists in the guinea pig lung.

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