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The role of endothelin-1 as a mediator of the pressure response after air embolism in blood perfused lungs

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Abstract *Objective*: It is well known that lung embolism is associated with an increase in pulmonary vascular resistance. Since the mechanisms of pulmonary vascular reactions during embolism are still unclear, the aim of this study was to investigate the potential involvement of endothelin-1 (ET-1) and thromboxane A_2 (TXA_2) as mediators of the pulmonary artery pressure (PAP) increase after embolism using the selective ET_A receptor antagonist LU135252 [1], the ET_B receptor antagonist BQ788 [2], and the cyclooxygenase inhibitor diclofenac. *Design*: Prospective experimental study in rabbits. *Setting*: Experimental laboratory in a university teaching hospital. *Subjects*: 36 adult rabbits of either sex. *Interventions*: The experiments were performed in 36 isolated and ventilated rabbit lungs which were perfused with a buffer solution containing 10 % of autologous blood. Embolism was induced by the injection of 0.75 ml air into the pulmonary artery. *Measurements and results*: PAP and lung weight, reflecting edema formation, were continuously recorded. Perfusate samples were drawn intermittently to determine TXA_2 and ET-1 concentrations. Air injection resulted in an immediate increase in PAP up to 22.8 ± 1.4 mm Hg at 2.5 min (control, $n = 6$), which was parallelled by an enhanced generation of TXA_2. No relevant edema formation occurred during the observation period. Pretreatment with the ET_A receptor antagonist LU135252 significantly reduced the pressure reaction after air embolism ($p < 0.001$) whereas the ET_B receptor antagonist BQ788 ($n = 6$) was without marked effects. The administration of diclofenac ($n = 6$) did not alter the PAP increase 2.5 min after embolism, but significantly reduced the pressure reaction during the further observation period ($p < 0.001$). The application of LU135252 and diclofenac together ($n = 6$) also significantly reduced the PAP increase from 2.5 min during the total observation period ($p < 0.001$). *Conclusions*: The acute pressure reaction after air embolism is mainly mediated via ET-1 by an ET_A receptor related mechanism. TXA_2 seems to maintain this reaction for a longer time.

Key words Air injection · Embolism · Endothelin · Thromboxane · Vascular resistance

Introduction

Since endothelin-1 (ET-1) was first described in 1988 [1], the effects of this potent vasoconstrictor have been under investigation. The peptide's activities are mediated through specific receptors, which can be classified by their different affinity to ET-1 and ET-3. So far now the DNA sequences of an ET_A receptor and an ET_B receptor subtype have been analyzed [2, 3]. Increasesd ET-1 levels were found in pulmonary diseases such as primary pulmonary hypertension [4], asthma and sepsis [5–7]. The aim of this study was to examine the potential involvement of ET-1 as a mediator of the acute pressure reaction after air embolism. For this purpose, the effects of the ET_A receptor antagonist LU135252 [8, 9] and the ET_B receptor antagonist BQ788 [10] on vascular resistance after air injection were tested in the isolated, blood perfused rabbit lung. The effects of the simultaneous application of LU135252 and diclofenac were additionally analyzed. There is some evidence that the vascular reactions of ET-1 are in part mediated via the activation of other mediator systems [11, 12]. The activation of the cyclooxygenase pathway, resulting in enhanced thromboxane A_2 (TXA_2), is probably involved in the mediation of ET-1 induced vasoconsriction [13]. To analyze the potential role of TXA_2 during the vascular reactions after air injection, the cyclooxygenase inhibitor diclofenac was used in our model.

Materials and methods

The lung model

Rabbits of either sex weighing 2900 ± 185 g (mean $\pm$ SD) were anesthetized with pentobarbital sodium (60–80 mg/kg) and anticoagulated with heparin-sodium (1000 IE/kg body weight). After tracheostomy the animals were mechanically ventilated via a tracheal tube. After a thoracotomy, the pulmonary artery was cannulated and immediately perfused with saline bovine serum albumin (BSA) solution. The lungs were removed from the thorax [14, 15]. The isolated lungs were suspended from an electronic weight balance (Hottinger, Baldwin Messtechnik Type U1, Darmstadt, Germany) in a temperature-controlled (37 °C) and humidified chamber. Ventilation of the lungs was performed with 4% CO_2 in air (frequency 25/min, tidal volume 30 ml, PEEP 0.5–1.0 cm H_2O). The pulmonary arterial pressure (PAP), airway pressure (AP) and weight of the isolated lungs were recorded continuously by means of pressure and weight transducers. Due to constant perfusion flow, the alterations of perfusion pressure directly reflect alterations of pulmonary vascular resistance. Intermittently, samples of perfusate were taken for calculations of pO_2, pCO_2 and O_2 saturation (ABL 330, Radiometer Copenhagen) and determinations oncotic pressure (Onkometer BMT 921, Dr. Karl Thomae GmbH, Biberach, GERMANY). Initially the lungs were perfused with saline BSA solution using low flow rates in the opened circulatory system. The perfusion fluid was then exchanged for fresh buffer via two separate perfusion circuits 2 min after the beginning of the extracorporeal circulation and again after 30 min, when the flow was increased up to 200 ml/min. Subsequently,

20 ml of autologous blood were added to the perfusate within 10 min.

During the whole experiment the flow was maintained at 200 ml/min. The addition of blood to the saline solution did not alter the PAP or the lung weight. During perfusion the integrity of the microcirculation was maintained for more than 5 h in our model. Homogenous capillary organ perfusion and the absence of structural endothelial damage (e.g., vacuolization, mitochondrial disintegration or the hydropic swelling of endothelial cells) could be verified by light and electronmicroscopic controls. No relevant alterations, in terms of vascular tone ($<$ ± 2 mmHg), permeability (weight increase < 1.5 g) or mediator release, occurred during this observation period. The entry criteria for acceptance in the present study were that the lungs had a homogenous white appearance without signs of hemostasis or edema formation (weight gain 0 g/min), and without changes in pulmonary vascular pressure ($\leq$ ± 1 mmHg) during the 30 min equilibration period.

Experimental protocol

Thirty-six lung preparations were randomly assigned to six groups containing six lungs each. Six lungs without intervention served as a control group. Following a 30-min equilibration period, lung embolism was induced by an injection of 0.75 ml air into the pulmonary artery (reference, $n = 6$). In the other experimental groups either LU135252 (10^{-6} M, $n = 6$), or BQ788 (10^{-6} M; $n = 6$), or diclofenac (10 µg/ml, $n = 6$) or LU135252 and diclofenac (10^{-6} M and 10 µg/ml, respectively, $n = 6$) were added to the perfusate 10 min prior to air injection. Immediately before and at defined time points (2.5, 5, 10, 15, 30, 60 min) after embolism perfusate samples were taken to determine ET-1 and TXA_2 concentrations.

Enzyme-linked immunosorbent assay of ET-1

Endothelin-1 was assayed from 100 µl of saline solution by enzyme immunoassay. The cross-reactivity of anti-ET-1 antibody was 100% with ET-1, more than 100% with ET-2, less than 0.001% with ET-3, 0.07% with big ET-1, and less than 0.0006% with Sarafotoxin 6b.

Radioimmunoassay of TXB_2

Thromboxane B_2 (TXB_2) was assayed serologically from 100 µl of recirculating buffer solution as a stable hydrolysis product of thromboxane A_2 by radioimmunoassay according to a method described by Peskar [16]. Radioactivity was quantified with a Philips PW 4700 liquid scintillation counter. The results were obtained by standard dose-response curves. The cross-reactivity of TXB_2 antiserum with prostaglandin D_2 was 2.7%, and 0.05% with 6-keto $PGF_1\alpha$, PGE_2, PGE_1, $PGF_1\alpha$, 13,14-dihydro-15-keto PGE_2, and 13,14-dihydro-15-keto $PGE_2\alpha$.

Materials

This study was approved by the Animal Subject Protection Committee of the University of Giessen. The care and handling of animals conformed to the Guiding Principles in the Care and Use of Animals as approved by the Council of American Physiologic Society.

Saline solution contained 2.4 mmol/l calcium chloride-dihydrate, 1.3 mmol/l, 4.3 mmol/l potassium chloride, 1.1 mmol/l potas-

Fig. 1 Changes in pulmonary arterial pressure (PAP) after air injection (0.75 ml) in untreated controls (n = 6) and in groups pretreated with the ET_A receptor antagonist LU135252 (10^{-6} M; n = 6) or with the ET_B receptor antagonist BQ788 (10^{-6} M; n = 6). The PAP increase after air embolism was only significantly reduced by LU135252. Data are presented as mean values ± standard error of the mean (SEM). **p < 0.01; ***p < 0.001 (ANOVA)
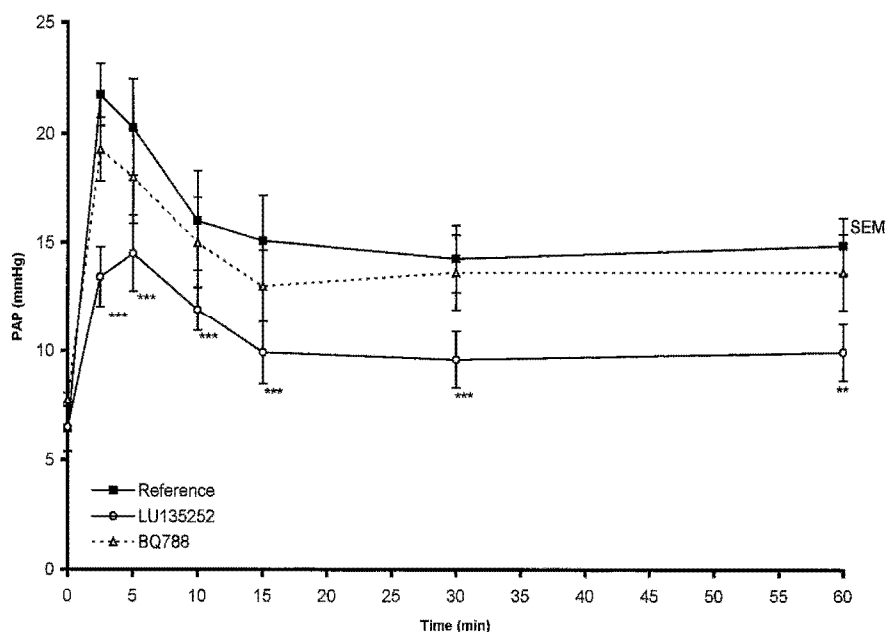
sium hydrogen phosphate, 125 mmol/l sodiumchloride, 2.5 g/l glucose and 1 mg/ml BSA (bovine serum albumin, Serva, Heidelberg, GERMANY). To adjust the pH to 7.4, $NaHCO_3$ 8.4% was used. Twenty milliliters of autologous blood were acquired after ligation and cannulation of the inferior vena cava. Diclofenac-sodium (Voltaren) was purchased from Ciba-Geigy (Wehr, GERMANY). Based on dose response studies, diclofenac was given in a concentration (10 μg/ml) which has been shown to inhibit the generation of cyclooxygenase products completely. A complete blockade of the cyclooxygenase pathway was achieved when diclofenac was added to the perfusion fluid 3 min prior to direct stimulation of the arachidonic acid metabolism by injection of calcium ionophore or arachidonic acid (unpublished data). The selective ET_A receptor antagonist LU135252 was a generous gift from Knoll AG (BASF Pharma, Ludwigshafen, GERMANY). LU135252 is a substituted propionic acid derivate with ET_A receptor affinity in the low nmol/l range [8, 9]. The dose of LU135252 (10^{-6} M) used was based on dose response studies (10^{-6}-10^{-9} M) which showed a complete inhibition of vascular reactions after injection of ET-1 in the concentration (10^{-8} M) used in our model (unpublished data). BQ788 was obtained from Alexis (Läufelfingen, Switzerland). The dose of BQ788 (10^{-6} M) used was based on previous publications [10, 17].

The doses of the antagonists LU135252, BQ788 and diclofenac were tested in pilot studies without any additional intervention. In these experiments they did not alter PAP, thromboxane A_2 levels and lung weight during the observation period of 210 min.

Statistical analysis

The data are presented as the mean ± standard error of the mean (SE). Differences between the groups were tested by one-way analysis of variance (ANOVA) followed by Scheffé's multiple range test (Statgraphics plus for Windows). Statistical significance was accepted at p less than 0.05.

Results

Pulmonary vascular reactions after air embolism

In the control group, lung weight was unaltered during the observation period (Δweight = ± 1.2 g). PAP remained between 7.5 ± 1.1 mmHg (baseline) and 10.2 ± 1.2 mmHg. Air injection (0.75 ml) resulted in an immediate increase in PAP (21.78 ± 1.4 mmHg) after 2.5 min (p < 0.01 vs control group). Thirty minutes after the induction of embolism, the PAP returned nearly to baseline values (Fig. 1). Before, and 60 min after, air injection there was no significant difference between the control and reference groups. No signs of edema formation occurred during the observation period of 60 min. The PAP increase was parallelled by a generation of TXA_2 (from 120 ± 45 pg/ml at 0 min to 980 ± 150 pg/ml at 5 min, p < 0.01 vs 0 min) (Fig. 2). ET-1 was not detectable in the perfusate.

Effects of the endothelin receptor antagonists LU135252 and BQ788 on PAP after air injection

Pretreatment with the ET_A receptor antagonist LU135252 (10^{-6} M) significantly reduced the PAP increase after air embolism (p < 0.001). The pressure reached only a value of 13.4 ± 1.4 mmHg at 2.5 min (21.8 ± 1.4 mmHg in the reference group) and 14.5 ± 1.8 mmHg at 5 min (20.3 ± 2.2 mmHg in the reference group). The PAP returned to baseline values 15 min after air injection (9.9 ± 1.4 mmHg vs 15.1 ± 2.1 mmHg in the reference group; p < 0.001) (Fig. 1). No significant difference in PAP occurred be-
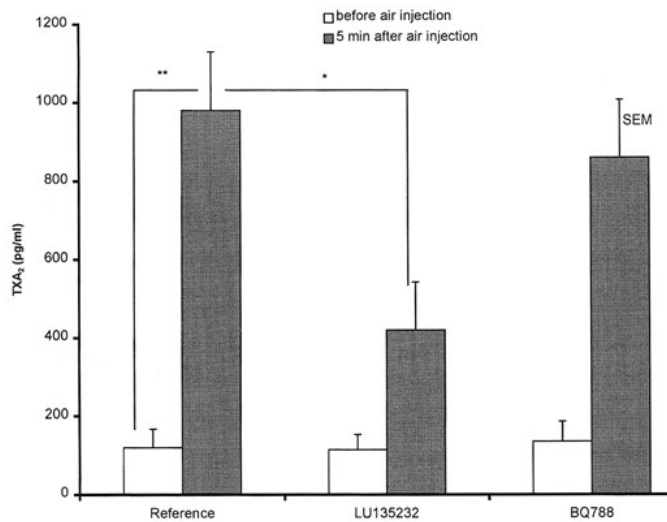
Fig. 2 Thromboxane A_2 (TXA_2) concentrations in the perfusate after air injection (0.75 ml; control; $n = 6$). The generation of TXA_2 after air embolism was significantly reduced by the ET_A receptor antagonist LU135252 (10^{-6} M; $n = 6$) whereas the ET_B receptor antagonist BQ788 (10^{-6} M; $n = 6$) was without marked effects. Data are presented as mean values ± standard error of the mean (SEM). $*p < 0.05$; $**p < 0.01$ (ANOVA)

tween the LU135252 and the control groups from 15 min until the end of the observation period. The generation of TXA_2 was also reduced as compared to the reference group (420 ± 123 pg/ml; $p < 0.05$) (Fig. 2).

The administration of the ET_B receptor antagonist BQ788 (10^{-6} M) did not reduce the vascular pressure response after embolism. A PAP maximum of 19.3 ± 1.5 mmHg at 2.5 min was recorded (21.8 ± 1.4 mmHg in

the reference group) and at 30 min the PAP reached 13.6 ± 1.7 mmHg (14.3 ± 1.6 mmHg in the reference group) (Fig. 1). Furthermore, the generation of TXA_2 was not influenced by BQ788 (860 ± 147 pg/ml) (Fig. 2).

## PAP after pretreatment with diclofenac

The cyclooxygenase inhibitor diclofenac (10 μg/ml) was used to quantify the potential involvement of TXA_2 in the pressure reaction after air injection. Pretreatment with diclofenac did not significantly attenuate the initial pressure response to air embolism (16.2 ± 0.7 mmHg at 2.5 min) but significantly reduced the PAP in the further observation period from 2.5 min until 60 min after air embolism (11.2 ± 0.8 mmHg; $p < 0.001$ vs reference). PAP baseline values were reached at 10 min (9.4 ± 1.1 mmHg; $p < 0.001$ vs reference) (Fig. 3).

Effects of the simultaneous administration of LU135252 and diclofenac on PAP after embolism

The simultaneous administration of LU135252 (10^{-6} M) and diclofenac 10 μg/ml significantly reduced the PAP maximum at 2.5 min (13.6 ± 0.6 mmHg; $p < 0.001$ vs reference). At this time point, the PAP was in the same range as had been recorded for the LU135252 group (13.4 ± 1.4 mmHg). Until the end of the observation period PAP remained significanty reduced (9.0 ± 0.8 mmHg; $p < 0.001$).

Fig. 3 Changes in pulmonary arterial pressure (PAP) after air injection (0.75 ml) in untreated controls ($n = 6$) and in lungs pretreated with the cyclooxygenase inhibitor diclofenac (10 μg/ml; $n = 6$) or a combination of LU135252 (10^{-6} M) and diclofenac (10 μg/ml; $n = 6$). 2.5 min after air embolism, the PAP reduction in the LU135252 and diclofenac groups was significant as compared to the controls, whereas the diclofenac group reached significant differences at 5 min. Data are presented as mean values ± standard error of the mean (SEM). $***p < 0.001$ (ANOVA)
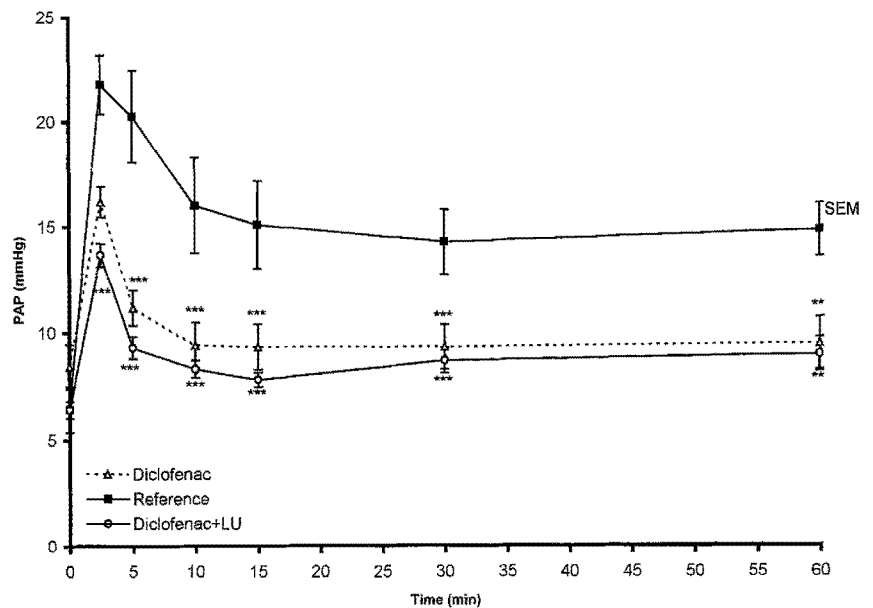
Discussion

Many clinical practices, such as cardiopulmonary bypass and thoracic surgery, are associated with the risk of air embolism [18, 19]. It is well known that air injection into a central vein is followed by a massive increase in PAP [20–22], as was seen in the present study. The mechanism of PAP increase after air embolism has been described as an interaction of mechanical vascular obstruction by the gas followed by the release of mediators [22]. The aim of our study was to investigate whether or not ET-1 is one of those mediators. Reviewing the data in the literature, it is still unclear whether ET-1 is related to the pressure reaction following air injection. Some data point out the importance of ET-1 as an mediator of the PAP increase after embolism [23] but reports to the contrary also exist [24]. In the present study, the pulmonary vascular reactions after air embolism were investigated in a rabbit lung model perfused with a saline solution enriched with 10 % of autologous blood.

Air injection into the pulmonary artery was followed by an immediate PAP increase which was parallelled by the generation of TXA_2. Pretreatment with the ET_A receptor antagonist LU135252 significantly reduced the pressure reaction after air embolism, while the ET_B receptor antagonist BQ788 was without any effect. The effect of an ET_A receptor antagonist on PAP after embolism was also supported by pilot experiments using the selective ET_A receptor antagonist BQ123 (unpublished data). The hypothesis of ET-1 related PAP increase after gas injection into the pulmonary artery has been supported by Wang et al. [23]. They were able to demonstrate increased ET-1 levels after continuous air infusion into the pulmonary artery of isolated rat lungs. In the present study ET-1 was not detectable in the perfusate. Since ET-1 is released in small amounts as a paracrine substance which is diluted in 200 ml of perfusate, it is not surprising that ET-1 was not detectable in our setting. Another fact is the well known clearing capacity of the pulmonary circulation, which is able to clear about 85 % of ET-1 with the first pass [25], especially in the presence of granulocytes with their high potency to degrade ET-1 by proteases [26]. Although investigators believe that cells do not store ET-1 [27], there is some evidence that endothelial cells can release big ET-1, which may be converted to active ET-1 by granulocytes. The postulated cooperative ET-1 synthesis between endothelial cells and granulocytes is supported by investigations in which granulocytes were found to induce endothelin-1 mRNA expression in vascular endothelial cells [28]. The cytosolic fraction of granulocytes is also able to convert big ET-1 to the active ET-1 [29, 30] by a cytosolic neutral protease [31].

The ET-1 induced effects seem to be mediated via ET_A receptors since the ET_A receptor antagonist LU135252 was found to reduce the pressure reaction after air injection. ET_A receptors have been described as the predominant ET receptor subtype in pulmonary circulation [32, 33]. Since ET_B receptor related pulmonary vasoconstriction has also been observed [34, 35], the effects of the ET_B receptor antagonist BQ788 on the PAP increase due to air embolism were investigated. The ET_B receptor antagonist BQ788 failed to inhibit the pressure reaction after air embolism. From the present data we conclude that ET-1 induced vasoconstriction in pulmonary circulation is mainly mediated via ET_A receptors. This hypothesis was supported by studies demonstrating no effects of the ET_B receptor agonist sarafotoxin S6c on pulmonary vessels [36, 37]. On the other hand, the inhibition of ET-1 related PAP increase was achieved using selective ET_A receptor antagonists [38, 39].

In the present study a marked increase in TXA_2 concentrations was observed after air embolism. In accordance with previous publications [23], the crucial role of TXA_2 as a mediator of the pressure reaction due to air injection was evidenced by increased TXA_2 concentrations in the perfusate and the significantly reduced PAP levels after pretreatment with the cyclooxygenase inhibitor diclofenac. The PAP increase was significantly reduced by the ET_A receptor antagonist LU135252 during the whole experiment. Furthermore, LU135252 markedly reduced the enhanced TXA_2 generation after embolism. In contrast to this, diclofenac only attenuated the sustained pressure reaction. From the current data it can be postulated that the release of ET-1 may activate the cyclooxygenase pathway resulting in increased TXA_2 levels. This hypothesis is supported by the finding that the simultaneous administration of LU135252 and diclofenac significantly reduced the initial pressure increase as observed in the LU135252 group, while PAP levels were similar to those in the diclofenac group during the further observation period. Furthermore, the activation of TXA_2 generation by ET-1 has also been previously reported [40].

In summary we conclude that the PAP increase after air embolism in the lung is mainly mediated by ET-1 via ET_A receptors. The activation, probably ET-1 related, of the cyclooxygenase pathway resulting in a generation of TXA_2 seems to be essentially involved in the pressure reaction due to air injection.

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