ET-1-induced pulmonary vasoconstriction shifts from ET<sub>A</sub>-to ET<sub>B</sub>-receptor-mediated reaction after preconstriction

JOACHIM SCHMECK, HEIDI GLUTH, NICOLAS MIHALJEVIC, MICHAEL BORN, MARTINA WENDEL-WELLNER, AND PETER KRAFFT
Department of Anesthesiology and Operative Intensive Care Medicine, University Hospital Mannheim, University of Heidelberg, 68135 Mannheim, Germany

Schmeck, J. ochaim, Heidi Gluth, Nicolas Mihaljevic, Michael Born, Martina Wendel-Wellner, and Peter Kraft. ET-1-induced pulmonary vasoconstriction shifts from ET<sub>A</sub>-to ET<sub>B</sub>-receptor-mediated reaction after preconstriction. J. Appl. Physiol. 87(6): 2284–2289, 1999.—Endothelin-1 (ET-1) has been reported to induce pulmonary vasoconstriction via either ET<sub>A</sub> or ET<sub>B</sub> receptors, and vasorelaxation after ET-1 injection has been observed. Our study investigated the effects of ET-1 in isolated rabbit lungs, which were studied at basal tone (part I) and after preconstriction (U-46619; part II). Pulmonary arterial pressure (PAP) and lung weight gain were monitored continuously. In part I, ET-1 (10<sup>-8</sup> M; n = 6; control) was injected after pretreatment with the ET<sub>A</sub>-receptor antagonist BQ-123 (10<sup>-6</sup> M; n = 6) or the ET<sub>B</sub>-receptor antagonist BQ-788 (10<sup>-6</sup> M; n = 6). The same protocol was carried out in part II after elevation of pulmonary vascular tone. ET-1 induced an immediate PAP increase (ΔPAP 4.3 ± 0.4 mmHg at 10 min) that was attenuated by pretreatment with BQ-123 (P < 0.05 at 10 min and P < 0.01 thereafter) and that was more pronounced after BQ-788 (P < 0.01 at 10 min and P < 0.001 thereafter). In part II, ET-1 induced an immediate rise in PAP with a maximum after 5 min (ΔPAP 6.3 ± 1.4 mmHg), leveling off at ΔPAP 3.2 ± 0.2 mmHg after 15 min. Pretreatment with BQ-123 failed to attenuate the increase. BQ-788 significantly reduced the peak pressure at 5 min (0.75 ± 0.4 mmHg; P < 0.001) as well as the plateau pressure thereafter (P < 0.01). We conclude that ET-1 administration causes pulmonary vasoconstriction independent of basal vascular tone, and, at normal vascular tone, the vasoconstriction seems to be mediated via ET<sub>A</sub> receptors. BQ-788 treatment resulted in even more pronounced vasoconstriction. After pulmonary preconstriction, ET<sub>A</sub> antagonism exerted no effects on PAP, whereas ET<sub>B</sub> antagonism blocked the PAP increase. Therefore, ET-1-induced pulmonary vasoconstriction is shifted from an ET<sub>A</sub>-to ET<sub>B</sub>-mediated mechanism after pulmonary vascular preconstriction.

BQ-123; BQ-788; endothelin; preconstriction


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VARIOUS STUDIES investigated the mechanisms of endothelin-1 (ET-1)-induced actions in pulmonary vessels. ET-1 is one of the most important vasoconstrictors in the pulmonary circulation (44), the effects of which are mediated via specific receptors, classified as ET<sub>A</sub> and ET<sub>B</sub> receptors (34, 35). ET<sub>A</sub>-receptor-mediated vasoconstriction has been shown in pulmonary arteries (1, 6), but controversy exists concerning the ET<sub>B</sub>-receptor-related vascular effects (22, 26, 30). Furthermore, ET-1-induced vasorelaxation has been described after pulmonary artery preconstriction (10). These controversial data indicate that the mechanisms of ET-1 actions in the pulmonary circulation are not definitively understood. There is evidence that ET-1 plays a key role in the pathophysiology of various diseases associated with increased pulmonary vascular tone. Elevated ET-1 levels have been observed during acute respiratory distress syndrome (8, 23), sepsis (20, 42), and primary pulmonary hypertension (14). Despite advances in intensive care medicine, these diseases are associated with high mortality rates, and new therapeutic strategies directly interfering with the underlying pathophysiological reactions are of major importance. ET-receptor antagonists might be beneficial for the therapy of pulmonary hypertension, but the exact mechanisms of the effects of ET-1 on the pulmonary circulation have to be elucidated first.

Our hypothesis was that the effects of ET-1 on pulmonary arterial pressure (PAP) depend on individual vascular tone. The purpose of the present study, therefore, was to investigate the effects of ET-1 on PAP in lungs with normal basal vascular tone or after pulmonary artery preconstriction with particular respect to the ET receptors involved. Therefore, ET-1 actions on untreated lungs were investigated in part I of the study. To analyze the receptor subtype mediating ET-1 effects, the selective ETA-receptor antagonist cyclo D-aspartyl-L-propyl-D-valyl-L-leucyl-D-tryp- tophyl (BQ-123) (17) and the selective ETB-receptor antagonist N-[N-[[(2,6-dimethyl-1-piperidinyl) carbonyl]-4-methyl-L-leucyl]-1-(methoxy carbonyl)-D-tryptophyl]-o-norleucine monosodium (BQ-788) (18) were administered before the ET-1 challenge. In part II of the study, the corresponding procedure was carried out after pulmonary artery preconstriction.

MATERIALS AND METHODS

Lung Model

The techniques of preparing and perfusing isolated rabbit lungs have been previously described in detail (21, 37). Rabbits of either sex weighing 2,900 ± 185 (SD) g were anesthetized with pentobarbital sodium (60–80 mg/kg) and anticoagulated with heparin-sodium (1,000 IE/kg body wt). Isolated lungs, suspended from an electronic weight balance (Höttinger, Baldwin Messtechnik Type U1, Darmstadt, Germany) in a temperature-controlled (37°C) and humidified chamber, were perfused with cell and plasma-free Krebs-Henseleit-hydroxy-ethyl-starch buffer solution (KHHB) or with saline solution enriched with 1 mg/ml BSA (for ET-1 measurements) at constant flow rates of 200 ml/min in a recirculating system (circulating volume 200 ml). Ventilation...
was performed with 4% CO₂ in air (frequency 25 breaths/min; tidal volume 30 ml; positive end-expiratory pressure 0.5–1.0 cmH₂O). PAP, airway pressure, and weight of the isolated lung were recorded continuously by means of pressure and weight transducers. Because of constant perfusion flow, alterations of perfusion pressure directly reflect alterations of pulmonary vascular resistance. Intermittently, perfusate samples were taken for measurements of Po₂, P CO₂, O₂ saturation (ABL 330, Radiometer, Copenhagen, Denmark), and oncotropic pressure (Onkometer BMT 921, Dr. Karl Thomae, Biberach, Germany).

Initially, the lungs were perfused with KHHB solution by using low flow rates in the opened circulatory system to remove residual blood from the vascular bed. 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 the flow was increased to 200 ml/min (over 30 min). The KHHB perfusion is able to maintain the integrity of the microcirculation for >5 h in our model. Homogenous capillary organ perfusion and absence of structural endothelial damage (e.g., vacuolization, mitochondrial disintegration, or hydropic swelling of endothelial cells) could be verified by light- and electron-microscopic controls. No relevant alterations, in terms of vascular tone (less than ±2 mmHg), permeability (wt increase <1.5 g), or mediator release occurred during this observation period. Furthermore, endothelial function remained unchanged (21). Entry criteria for acceptance in the present study were that the lungs had a homogenous white appearance without signs of hemostasis or edema formation (wt gain 0 g/min) and without changes in vascular resistance (less than or equal to ±1 mmHg) during the 30-min equilibration period.

Experimental Protocol

Forty-eight lung preparations were randomly assigned to eight groups (6 lungs/group). Furthermore, six experiments without intervention for each part of the study served as the sham group.

In part I of the study ET-1 (10⁻⁸ M, control) was injected into the pulmonary artery after the steady-state period. The initial PAP before the application of ET-1 was set at zero. Additionally, the ETₐ-receptor antagonist BQ-123 (10⁻⁶ M), the ETₐ-receptor antagonist BQ-788 (10⁻⁶ M), or a combination of both was given 10 min before the ET-1 application.

In part II of the study, pulmonary vessels were preconstricted by the infusion of the thromboxane analog U-46619 (50 pmol/min) before ET-1 (10⁻⁸ M, control) was injected. The initial PAP before the application of ET-1 was set at zero. BQ-123 (10⁻⁶ M), BQ-788 (10⁻⁶ M), or a combination of both was injected 10 min before to investigate ET-receptor-mediated mechanisms.

This study was approved by the Animal Subject Protection Committee of the University of Heidelberg. The care and handling of animals conformed to the Guide for the Care and Use of Laboratory Animals [DHEW Publication No. (NIH) 86–23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20892] as approved by the Council of the American Physiological Society.

Materials

Perfusion consisted of a Krebs-Henseleit buffer solution with additional poly(0-2-hydroxy-ethyl)-starch (Haes-steril 10%, Fresenius, Bad Homburg, Germany) to maintain a colloidal oncotic pressure between 23 and 25 mmHg, yielding the following final concentrations (in mM): 138 Na⁺; 4.5 K⁺; 1.33 Mg²⁺; 135 Cl⁻; 2.38 Ca²⁺; 12 glucose; and 12 HCO₃⁻, as well as 50 g/l starch. The osmolality was ~330 mosmol/kg (Mikro-Osmometer, Roehling Messtechnik, Berlin, Germany). The perfusate pH was adjusted to 7.4 with 1 M NaHCO₃ shortly before cannulation.

ET-1 (155-001-P001), U-46619 (340-015-M005), BQ-123 (155-004-P005), and BQ-788 (155-020-M001) were obtained from Alexis (Grüneberg, Germany). The dose of U-46619 (50 pmol/min) was used to preconstrict the pulmonary circulation was based on previous publications (1, 9). The integrity of endothelial function was demonstrated by using acetylcholine, which was able to induce vasodilatation in U-46619-treated lungs (J. Schmedk, C. Konrad, S. Schöffel, M. Wendel-Weliner, H. Gluth, T. Koch, and P. Krafft, unpublished observations). The selective ETₐ-receptor antagonist BQ-123 is a synthetic analog based on an ETₐ-receptor antagonist isolated from Streptomyces misakiiensis with a high binding affinity (IC₅₀ 7.3 nM) to the ETₐ receptor (16, 17). The dose of BQ-123 (10⁻⁶ M) used was based on previous publications from our group (37, 38) and other investigators (2, 5). BQ-788 is a norleucine derivative with a high affinity to the ETₐ receptor (IC₅₀ 1.2 nM) (13, 18). The dose of BQ-788 was used based on previous publications (13, 38).

Statistical Analysis

Data are presented as means ± SE. Differences between the groups were tested by ANOVA, followed by Scheffé's multiple-range test (Statgraphics Plus for Windows). A t-test for paired samples was used to analyze differences within groups. Statistical significance was accepted at P < 0.05.

RESULTS

Part I: Effects of ET-1 on Pulmonary Vessels With Normal Basal Vascular Tone

Pilot experiments. To exclude nonspecific PAP changes during the experiments, lungs without any intervention served as the sham group (n = 6). PAP reached 7.2 ± 0.4 mmHg at the beginning of the experiments (time 0) and 7.5 ± 0.2 mmHg 60 min thereafter. The application of ET-1 (n = 6) did not alter the PAP course (7.1 ± 0.2 mmHg at 0 min; 7.4 ± 0.3 mmHg at 60 min). Furthermore, PAP remained unchanged after the injection of BQ-788 (n = 6; 7.3 ± 0.4 mmHg at 0 min; 7.6 ± 0.3 mmHg at 60 min).

In the control group, the injection of ET-1 resulted in an immediate PAP increase, with a maximum observed after 15 min (ΔPAP 5.8 ± 1.2 mmHg). Thereafter, a sustained PAP increase was found throughout the entire study period of 60 min (up to 7.3 ± 1.8 mmHg; Fig. 1). No signs of lung weight gain reflecting edema formation occurred during the observation period.

Pretreatment with the ETₐ-receptor antagonist BQ-123 significantly reduced the PAP increase after ET-1 treatment beginning at 10 min (ΔPAP 2.8 ± 0.6 vs. 4.3 ± 0.6 mmHg; P < 0.05) until the end of the experiments (ΔPAP 3.7 ± 0.3 mmHg; P < 0.01; Fig. 1). The reduction of PAP did not reach levels in sham-operated lungs (P < 0.01 from 10 to 60 min). On the contrary, ΔPAP was potentiated when the ETₐ-receptor antagonist BQ-788 was given (Fig. 1). Fifteen minutes after ET-1 injection, the PAP increase was 18.5 ± 1.1 mmHg compared with 5.8 ± 1.2 mmHg in the control group (P < 0.001) and continued to rise until 60 min.
(ΔPAP 24.6 ± 1.0 vs. 7.3 ± 1.8 mmHg in the control group; P < 0.001). Statistical significance was also reached compared with the BQ-123 group (P < 0.01 at 10 min and P < 0.001 until the end of the experiments). The concurrent administration of BQ-123 and BQ-788 was followed by results corresponding to those observed after BQ-123 treatment alone (ΔPAP 2.6 ± 1.1 mmHg at 10 min and 4.2 ± 1.1 mmHg at the end of the observation period).

Part II: Effects of ET-1 on Preconstricted Pulmonary Vessels

Pilot experiments. In sham-operated lungs (n = 6), PAP was raised to 18.2 ± 1.4 mmHg by the continuous infusion of the thromboxane analog U-46619. PAP remained on that high level during the observation period of 60 min (18.4 ± 0.8 mmHg). Neither the application of BQ-123 (18.6 ± 2.3 mmHg at 0 min; 18.9 ± 3.1 mmHg at 60 min) nor the injection of BQ-788 (18.5 ± 1.9 mmHg at 0 min; 19.0 ± 1.8 mmHg at 60 min) influenced PAP.

PAP was raised to 18.5 ± 2.1 mmHg by using U-46619. Corresponding to untreated pulmonary vessels, in preconstricted lungs ET-1 also induced a PAP increase. Maximum PAP increase was reached after 5 min (ΔPAP 6.3 ± 1.4 mmHg), followed by a PAP decrease to ΔPAP 2.7 ± 0.3 mmHg at 60 min (Fig. 2). Lung weight gain remained unchanged during the entire study period.

Pretreatment with BQ-123 did not significantly influence the PAP increase after ET-1 injection. At 5 min, ΔPAP reached 5.0 ± 0.6 mmHg and decreased to 1.1 ± 1.3 mmHg at 60 min (Fig. 2). The PAP increase after ET-1 injection was almost completely abandoned by BQ-788 added to the perfusate (Fig. 2). The PAP increase reached 0.8 ± 0.4 mmHg at 5 min (P < 0.001) and 0.1 ± 1.2 mmHg at 60 min (P < 0.01). At 5 (P < 0.001) and 10 min (P < 0.01), significant differences were also observed between the BQ-123 and the BQ-788 groups. There was no statistical difference in the sham-operated lungs. The concurrent administration of BQ-123 and BQ-788 did not result in any different results (ΔPAP at 5 min 2.5 ± 0.2 mmHg) compared with BQ-788 solely.

DISCUSSION

It has been postulated that ET-1 is essentially involved in the underlying pathophysiological process of certain pulmonary diseases associated with pulmonary hypertension, such as acute respiratory distress syndrome (32), endotoxin invasion (29), and primary pulmonary hypertension (19). The effects of ET-1 are mediated via specific receptors classified as ETA and ETB receptors (28). Pulmonary vasoconstriction seems to be mediated via the ETA receptor, which is the receptor predominantly expressed in the pulmonary circulation (15). However, further studies have also reported ETB-receptor-mediated PAP increase (41) and even ET-1-induced pulmonary vasodilation in spontaneously breathing animals (7, 10).

The present study was designed to investigate the effects of ET-1 on isolated rabbit lungs perfused with a cell- and plasma-free buffer. Our hypothesis was that ET-1 may exert different reactions depending on the individual contractile state of pulmonary circulation. The effects of ET-1 on pulmonary vessels with normal basal tone were tested in part I of the study. The selective ETA-receptor antagonist BQ-123 and the selective ETB-receptor antagonist BQ-788 were used to analyze ET-receptor-related effects. In part II of the study, the pulmonary circulation was preconstricted by using the thromboxane analog U-46619 before the ET-1 injection. BQ-123 and BQ-788 were also used to evaluate the mechanisms of ET-1-mediated actions.
Part I

The administration of ET-1 to isolated perfused rabbit lungs caused pulmonary vasoconstriction, independent of basal vascular tone. The finding of an ET-1-induced pressure increase in pulmonary vessels is in accordance with previous publications. ET-1-induced vasoconstriction has been described previously for isolated pulmonary artery preparations (3), organ preparations (33), as well as intact animals (24). Furthermore, a close correlation between circulating ET-1 levels and the extent of pulmonary hypertension has been observed in humans (4).

Pulmonary vasoconstriction at normal vascular tone seems to be mediated via ET A receptors and can be antagonized by ETA-receptor blockade. Similar results were obtained in rat lung preparations (1) and in beagles with pulmonary hypertension (31). To the contrary, pretreatment with BQ-788 resulted in even more pronounced vasoconstriction, indicating an inhibition of ETB-receptor-mediated vasodilation, which could probably be induced by ET-1. Enhanced PAP pressure response after ETB-receptor antagonism might be explained by the blockade of ETB-receptor-mediated vasodilator activities of ET-1. The release of the vasodilators prostacyclin (25) and nitric oxide (5, 27) might also be associated with the interaction between ET-1 and the ETB receptor. These reactions may serve as an internal feedback mechanism to avoid uncontrolled vasoconstriction after ET-1 release. Furthermore, ETB receptors are essentially involved in the metabolism of ET-1 (11). About 85% of circulating ET-1 is cleared by the lung during the first pass (28). From this point of view, the potentiation of ET-1-induced rise in PAP might be explained by higher ET-1 concentrations in the pulmonary circulation due to a reduced metabolism. An ETB-receptor-related PAP increase has also been shown in preliminary experiments by our group through the use of the selective ETB-receptor agonist sarafotoxin 6c (S6c) (unpublished observations).

Part II

ET-1 also increased PAP in preconstricted pulmonary vessels. Treatment with the ETA-receptor antagonist BQ-123 did not significantly affect the PAP pressure reaction, whereas an almost complete blockade of PAP response was observed after the administration of the ETB-receptor antagonist BQ-788, indicating ETB-receptor-mediated vasoconstriction. An ET-1-induced PAP increase has also been shown in lambs, with increased pulmonary blood flow after placement of a vascular shunt (43) and after pulmonary artery ligation (39). In contrast to this, ET-1-induced vasorelaxation has been described after preconstriction (9). The aforementioned different findings might be explained by differing severity of preconstriction before ET-1 treatment. Because the effects of ET-1 were studied after pretreatment with a thromboxane analog, we cannot exclude a different pattern induced by other vasoactive substances.

The possibility of ETB-receptor-mediated vasoconstriction has been discussed previously. In addition to ETA-receptor-related vasopressor effects, Fukoroda et al. (12) postulated a role for the ETB receptor in the mediation of ET-1-induced vasoconstriction in coronary and pulmonary vessels. ETB-receptor-mediated vasoconstriction has also been reported in rabbit pulmonary vessels (22). Sudjarwo et al. (40) postulated that a separate type of ETB receptor is responsible for the mediation of vasoconstriction. It has been hypothesized that two different types of ETB receptors exist: one mediating vasoconstriction and the other mediating vasodilation (32). The observation of an enhanced PAP increase after ETB-receptor blockade in part I of our
study, together with the inhibition of ET-1-induced vasoconstriction after ETA-receptor blockade in part II, seems to support this hypothesis.

In summary, we conclude that ET-1 administration to isolated perfused rabbit lungs induces pulmonary vasoconstriction independent of basal vascular tone. This PAP increase seems to be mediated via ETA receptors in lungs with normal vascular tone and via ETB receptors in lungs with preconstricted pulmonary vessels. Whether antagonism of the ETA-receptor-mediated vasorelaxation or reduced ET-1 metabolism is responsible for the potentiated pressure response after ETA-receptor blockade cannot be definitively answered by the present study. Enhanced PAP increase after ETB-receptor blockade together with the inhibition of ET-1-induced vasoconstriction after ETB-receptor blockade provide further evidence that two subtypes of ETB receptors exist, mediating either vasodilatation or vasoconstriction.

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Address for reprint requests and other correspondence: J. Schmeck, Dept. of Anesthesiology and Operative Intensive Care Medicine, Univ. Hospital Mannheim, Unv. of Heidelberg, Theodor-Kutzer-Ufer, 68135 Mannheim, Germany (E-mail: joachim.schmeck@anaes.ma.uni-heidelberg.de).

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