Pulmonary adrenomedullin counteracts deterioration of coronary flow and myocardial performance evoked by pulmonary endothelins in experimental acute respiratory distress syndrome

Karl Stangl, MD; Michael Laule, MD; Christoph Richter, MD; Verena Stangl, MD; Jonas Koch, MD; Öndar Göktas, MD; Gert Baumann, MD; Thomas Dschietzig, MD

Objectives: **We recently showed that pulmonary endothelins may affect coronary circulation under various experimental and clinical conditions. Here, we investigated the effect of pulmonary mediators on coronary tone in experimental acute respiratory distress syndrome. We focused particularly on pulmonary endothelin-1, a major vasoconstrictor in acute respiratory distress syndrome, and on adrenomedullin, a potent vasodilator that is up-regulated by inflammatory stimuli.**

Design: **Controlled experiment that used isolated organs.**

Setting: **Experimental laboratory.**

Subjects: **Wistar rats.**

Interventions: **The saline effluent from an isolated lung was used to serially perfuse the coronary vessels of an isolated heart. We compared serial perfusion after 2-hr pretreatment of lungs with vehicle or endotoxin (50 μg/mL), and we used the following drugs to elucidate the coronary response observed: the endothelin type A receptor antagonist BQ-123 (2 μM), the endothelin type B antagonist A-192621 (500 nM), the endothelin-converting enzyme inhibitor phosphoramidon (50 μM), the calcitonin gene-related peptide type-1 receptor antagonist hCGRP(8-37) (2 μM), and the adrenomedullin receptor antagonist hAM(22-52) (200 nM) (n = 6 each).**

Measurements and Main Results: **In controls, serial perfusion decreased coronary flow to 87 ± 3% of baseline ($p < .05$). BQ-123 and phosphoramidon prevented this effect, whereas blockade of endothelin type B and adrenomedullin-binding receptors had no effect. After endotoxin challenge, coronary flow significantly increased to 110 ± 2%. This response was augmented by BQ-123 (124 ± 2%) and phosphoramidon (123 ± 3%); A-192621 had no effect. Application of hCGRP(8-37) and hAM(22-52) significantly decreased coronary flow to 81 ± 3% and 88 ± 2%, respectively. Flow decrease after blockade of both adrenomedullin-binding receptors (73 ± 2%) significantly deteriorated peak left ventricular pressure, to 82 ± 6% of baseline; rate of pressure increase, to 81 ± 5%; and rate of pressure decline, to 77 ± 6%. Endotoxin pretreatment elevated pulmonary venous big endothelin-1 (three-fold), endothelin-1 (two-fold), and adrenomedullin (five-fold).**

Conclusion: **In experimental acute respiratory distress syndrome, pulmonary adrenomedullin—via calcitonin gene-related peptide type-1 receptor and adrenomedullin receptor—outweighs the coronary vasoconstrictor impact of pulmonary big endothelin-1 exerted via endothelin type A receptors after conversion to mature endothelin-1. The consequence is prevention of flow-related deterioration of myocardial performance. (Crit Care Med 2001; 29:1027–1032)**

KEY WORDS: coronary circulation; endothelins; adrenomedullin; pulmonary circulation; inflammation; contractile function

Lung tissue in general and pulmonary vasculature in particular produce a variety of vasoactive mediators, including arachidonic acid metabolites (1); endothelium-derived relaxing factor (nitric oxide) (2); atrial (3), brain (4), and C-type natriuretic peptides (5); endothelin (ET)-1 (6); and adrenomedullin (AM) (7).

Through release of these mediators into pulmonary circulation, the lungs may affect coronary vascular tone. We have recently demonstrated in experimental and clinical studies that pulmonary ETs—big ET-1 and ET-1—exert such an influence: namely, elevation of coronary tone under basal conditions (8), in experimental pulmonary embolism (9), and in severe human heart failure (10).

The manner in which this remote pulmonary effect on the coronary vascular bed is altered under conditions of acute respiratory distress syndrome (ARDS) is a matter of pathophysiological interest, because left ventricular failure is a common complication of ARDS. Different inflammatory stimuli have been reported to up-regulate vasoactive mediators in the lungs, including the vasoconstrictor ET-1 (11) and the vasodilatory peptide AM (12). Besides thromboxane, ET-1 constitutes a major mediator of pulmonary hypertension during ARDS (13). On the other hand, the previously mentioned up-regulation of pulmonary AM system by inflammatory stimuli might counteract ET-1 effects. AM—a 52 amino acid-peptide originally isolated from human pheochromocytoma (14)—is produced by endothelial cells, vascular smooth muscle cells, myocytes, and various other cell types. Apart from its vasodilatory action, AM shows natriuretic as well as antiproliferative effects, and it is currently regarded as one of the most important counterregulatory mediators in human heart failure (15).

In the present experimental study we addressed how endotoxin-treated lungs alter coronary tone, with particular attention to the contrary actions of pulmonary ETs and pulmonary AM.

From Medizinische Klinik und Poliklinik I (Kardiologie), Charité (Campus Mitte), Humboldt-Universität zu Berlin, Germany.

MATERIALS AND METHODS

The study conforms to guidelines published by the U.S. National Institutes of Health (16).

Model of Serial Lung-Heart Perfusion. Owing to difficulties involved in selectively focusing on pulmocoronary interactions in a complex *in vivo* model, we have developed a model of serial lung-heart perfusion. This model has been described in detail for rabbits (8), and in the present study we used it with the same techniques for Wistar rats (200–300 g body weight) with the exception that it was necessary for us to use hearts and lungs from distinct rats because of the 2-hr pretreatment of the lungs (see subsequent description of protocol). We validated in numerous control experiments in rats (n = 20) that no differences arise on comparison of serial saline lung-heart perfusions prepared from the same animal with those prepared from two different animals.

Isolated hearts and isolated lungs were prepared after intraperitoneal injection of pentobarbital sodium (50 mg/kg) with use of standard procedures. We perfused the hearts at constant pressure of 60 mm Hg with a modified Krebs-Henseleit solution (in mM: NaCl 116, KCl 4.0, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 2.5, glucose 10, HEPES 6; 37.0°C; pH = 7.35–7.40), equilibrated with 95% oxygen and 5% CO_2. Left ventricular pressures (LVP) were recorded with a fluid-filled latex balloon inserted through the mitral valve and attached to a pressure transducer. End-diastolic LVP was maintained between 3 and 6 mm Hg. Balloon pressures were differentiated electronically to yield rates of pressure increase and decrease (dP/dt) and heart rate (HR). An ultrasonic flow probe (Transsonic Systems, Fürstenfeldbruck, Germany) inserted in the aortic perfusion line measured the coronary flow (CF). A pressure transducer attached to the aortic perfusion cannula measured coronary perfusion pressure (CPP). Registration included HR, LVP, left ventricular peak dP/dt ($LVdP/dt_{max}$ and $LVdP/dt_{min}$), CF, and CPP. Only those hearts were accepted that achieved a contractile performance of >80 mm Hg LVP_{max} and >1500 mm Hg/sec $LVdP/dt_{max}$ and $LVdP/dt_{min}$.

Lungs were ventilated with a small-animal respirator (60 strokes/min, tidal volume 8–10 mL/kg body weight, 1 mm Hg positive end-expiratory pressure, gas mixture 21% oxygen, 5% CO_2, and 74% N_2). We performed perfusion with the Krebs-Henseleit buffer described previously. Lungs were suspended in a humidified chamber from a force transducer that monitored changes in lung weight. Within 20 mins, pulmonary flow was increased gradually and finally maintained at 14 mL/min. Only those lungs were selected for the present study that showed constant mean pulmonary arterial pressure (5–8 mm Hg, zero-referenced at hilum), constant peak inflation pressure of 7–10 mm Hg, practically no weight gain (<50 mg/hr), and no signs of hemorrhage, edema, or atelectasis.

The apparatus used in our investigation was manufactured by Hugo Sachs Electronics (March-Hugstetten, Germany). Modification of the device allows rapid switching from separate to serial perfusion of heart and lungs. With a switch, the amount of pulmonary venous effluent that equals coronary flow is rapidly conveyed to the heart apparatus, where it is oxygenated before becoming perfused through the coronary vessels.

Experimental Protocol—Serial Perfusion Experiments. During the first phase of these experiments, both organs were perfused separately. Initially, the lung was perfused in nonrecirculatory mode and controlled over 30 mins for constant mean pulmonary arterial pressure and weight. Lung perfusion subsequently was conducted in recirculatory mode over 2 hrs with circulating volume of 80 mL, in the presence of either lipopolysaccharide (LPS; 50 μg/mL) or vehicle alone (controls) (n = 6 for both). Finally, perfusion mode for the lung was switched back in both groups to nonrecirculatory perfusion with LPS-free buffer. Ten minutes after this switch, isolated hearts had already stabilized over 20 mins, and it was possible to conduct serial perfusion over 30 mins in both groups. At the beginning of serial perfusion, LPS content in pulmonary venous effluent of both groups was <1 ng/mL (LAL assay; BioWhittaker, Verviers, Belgium). By using this protocol we avoided any possible bias of CF by the direct vasodilatory effects of exogenous LPS known to occur in rats at concentrations ≥1 μg/mL (17).

Experimental ARDS. The LPS challenge in rats is a well-characterized ARDS model: First, rats—in contrast with other species—do not display relevant pulmonary vascular response to LPS. This phenomenon has been observed by several investigators *in vivo* (11) and *in vitro* (18). But, despite this lack of pulmonary vascular response, rats challenged with LPS *in vivo* develop relevant characteristics of ARDS, that is, pulmonary leukocyte accumulation, followed by lung damage and increased permeability (19). Because the endotoxin-induced pulmonary injury is mediated mainly by activated neutrophils (19), we documented no change in permeability in our model of blood-free perfusion, which confirms data reported by Uhlig et al (18). Hence, the lung model used in these experiments allows changes in pulmonary mediators to be observed without interference by mechanical damage or altered hemodynamics. On the other hand, functional consequences of these alterations can be assessed only in intact animals and are beyond the scope of this study.

Pharmacological Interventions During Serial Perfusion. Each of the following drugs was given to different subgroups (n = 6 for each subgroup) of both LPS and control preparations: BQ-123 (2 μM), an ET_A receptor-selective antagonist demonstrating an IC_{50} = 7.3 nM for ET_A receptors and an IC_{50} = 18 μM for ET_B receptors (20); the ET_B receptor-selective antagonist A-192621 (500 nM), characterized by potency of agonist values of 8.4 for ET_B receptors and 5.2 for ET_A receptors (21); phosphoramidon (50 μM), an inhibitor of ET-converting enzymes (ECE) with an IC_{50} = 1 μM for ECE-1 and an IC_{50} = 4 nM for ECE-2 (22); hCGRP(8-37) (2 μM), an antagonist of the calcitonin gene-related peptide (CGRP) type-1 receptor (CGRP-R1) demonstrating a potency of agonist value of 7.0 in rat coronary arteries (23); and hAM(22-52) (200 nM), an antagonist of the specific AM receptor, the IC_{50} of which is 70 nM in rat mesangial cells (24). The latter two antagonists were chosen for two reasons: first, because AM elicits its effects via the CGRP-R1 or the AM receptor, depending on the tissues being studied, and second, because several reports have indicated that AM action in rats is not in all cases sensitive to hCGRP(8-37) (25, 26). For these two reasons, we administered hCGRP(8-37) and hAM(22-52) alone and in combination.

Drugs were applied as coronary infusion, which in all cases commenced 15 mins before the switch from separate to serial perfusion and which we maintained throughout the experiment.

Control Experiments in Isolated Hearts. To compare the results obtained in serial perfusion experiments with effects of exogenous vasoactive mediators, and to verify the effectiveness of the different antagonists, isolated hearts received 30-min infusions of 5 pM rat ET-1 and big ET-1, as well as 100 pM rat AM (n = 6 each). Moreover, ET-1 and big ET-1 were administered in the presence of BQ-123, A-192621, and phosphoramidon, and AM was given in presence of hCGRP(8-37) and hAM(22-52) (n = 6 for each drug). All antagonists were applied 15 mins before infusion of vasoactive mediators began. In additional control experiments (n = 3 for each drug), we verified that none of the drugs interfered with CF or with cardiac contractility over 45 mins.

Drugs. Rat AM and hCGRP(8-37) were purchased from Bachem (Heidelberg, Germany). Rat ET-1, big ET-1, hAM(22-52), BQ-123, phosphoramidon, and *salmonella minnesota* LPS were from Sigma Chemical (Munich, Germany). A-192621 was a gift from Abbott Labs (Abbott, IL).

Determination of Vasoactive Peptides in Pulmonary Venous Effluent. The amount of pulmonary venous effluent not conveyed to the heart owing to the difference between coronary and pulmonary flow was sampled during serial perfusion according to the following protocol: sample 1, minutes 0–10; sample 2, minutes 11–20; sample 3, minutes 21–30. After admixture of 0.2 g% bovine serum albumine, samples were rapidly frozen in liquid nitrogen and stored at −70°C for subsequent determination of big ET-1, ET-1, and AM.

In additional experiments (n = 4 for both LPS and controls), we excluded the possibility that preceding recirculatory perfusion of the lungs alters pulmonary venous concentrations

of ET and AM during serial perfusion. Indeed, adopting the protocol described under Experimental Protocol—Serial Perfusion Experiments—with the exception that lung perfusion was exclusively conducted in nonrecirculatory mode—changed neither big ET-1, ET-1, nor AM values in pulmonary venous effluent (data not shown).

Radioimmunoassays for Big ET-1 and ET-1. The radioimmunoassay for rat big ET-1 has been established by Brunner et al. (27), as described previously. To summarize, a polyclonal antiserum against big ET-1 (1-39; rat) was raised in rabbits. Standard solutions of big ET-1 (1-39; rat) were used at 2–512 pg/assay; bound radioactivity was separated by using polyethylene glycol. The detection limit was ~2 pg per assay tube (~10% displacement of the radioactive tracer), and the IC_{50} value was 45 pg/tube. The cross-reaction of the antiserum was 16% with big ET-1 fragment 22-39 (bovine), and zero with rat ET-1, rat atrial natriuretic peptide (1-28), and rat angiotensin II (~0.1% at the IC_{50} concentration in each case).

The concentration of rat ET-1 was determined by radioimmunoassay by using a commercial kit (Peninsula, Belmont, CA) as described previously (28). The detection limit was ~0.15 pg/tube; the cross-reactivity of other ET isomers and big ET-1 in this assay was <5% and <37%, respectively, according to the supplier.

Radioimmunoassay for Adrenomedullin. Rat AM was determined by the use of a commercial radioimmunoassay kit (Phoenix Pharmaceuticals, Mountain View, CA). This kit (detection limit 0.3 pg/tube) demonstrates high selectivity for rat AM(1-50) (100%). With this kit, cross-reactivity for rat proadrenomedullin N-terminal 20 peptide, human amylin, and rat ET-1 is very low (<0.1%).

Statistical Analysis. Data are presented as mean ± SEM. Baseline values of isolated organs were compared by using the Kruskal-Wallis analysis of variance on ranks. Differences between groups over time were analyzed by a nonparametric analysis of variance for repeated measures (29). After global testing, we performed a multiple-comparison procedure with Bonferroni-Holm adjustment of p (30). An error probability of $p < .05$ was regarded as significant.

RESULTS

Serial Perfusion—Baseline

Immediately before beginning serial perfusion, all relevant variables were nearly identical in hearts of the control and the LPS groups: CF, 7.5 ± 0.5 vs. 7.7 ± 0.6 mL/min; HR, 322 ± 15 vs. 315 ± 20 beats/min; LVP_{max}, 95 ± 7 vs. 93 ± 8 mm Hg; $LVdP/dt_{max}$, 2004 ± 123 vs. 2103 ± 143 mm Hg/sec; and $LVdP/dt_{min}$, 1634 ± 119 vs. 1567 ± 133 mm Hg/sec. Also, control and LPS lungs exhibited similar mean pulmonary arterial pressure and weight gain at the end of 2 hrs of recirculatory perfusion (controls, 7.3 ± 0.5 mm Hg, +10 ± 12 mg; LPS, 7.5 ± 0.6 mm Hg, −1 ± 13 mg) and during nonrecirculatory perfusion immediately before serial perfusion (controls, 7.5 ± 0.5 mm Hg, +9 ± 11 mg; LPS, 7.4 ± 0.8 mm Hg, +2 ± 9 mg).

Serial Perfusion

Figure 1 shows the coronary flow response in the control and the LPS groups. In controls, CF reached a new steady state at 87 ± 3% of the baseline value after approximately 10 mins. In contrast, CF in the LPS group increased to 110 ± 2% of baseline rate. In both groups, neither HR nor contractile parameters changed significantly compared with baseline values (data not shown).

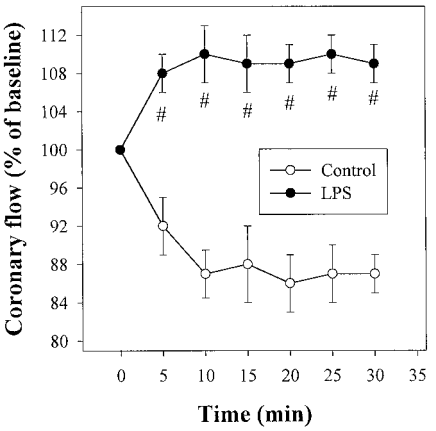

Figure 1. Coronary flow during serial perfusion of isolated hearts with effluent of control lungs (n = 6) and with effluent of lungs pretreated with lipopolysaccharide (*LPS*; 50 μg/mL) over 2 hrs (n = 6). Switch from separate to serial perfusion was performed at time zero (baseline). Data are given as percentage of baseline coronary flow. #$p < .05$, control vs. LPS.

Pharmacological Interventions During Serial Perfusion

Figure 2 shows CF during the various pharmacological interventions. None of the drugs or drug combinations influenced CF, HR, or contractile parameters during the 15-min pretreatment immediately before beginning serial perfusion (data not shown). In controls, the ET_A antagonist BQ-123 and the ECE inhibitor phosphoramidon completely inhibited the decrease in CF during serial perfusion, whereas A-192621 (data not shown), hCGRP(8-37), hAM(22-52), and the combination of the latter two drugs had no effect. In the LPS group, we observed the following coronary responses: Presence of BQ-123 and phosphoramidon significantly augmented flow increase, to 124 ± 2% and 123 ± 3% of baseline rate, respectively, compared with untreated preparations. Administration of A-192621 had no effect (data not shown). Application of hCGRP(8-37) and hAM(22-52) significantly decreased CF, with values recorded at 81 ± 3% and 88 ± 2%, respectively. Furthermore, combined application of the latter two drugs resulted in a significantly steeper decrease in CF compared with the single administrations: i.e., to 73 ± 2% of baseline. In this case, LVP_{max} (82 ± 6% of baseline), $LVdP/dt_{max}$ (81 ± 5%), and $LVdP/dt_{min}$ (77 ± 6%) deteriorated significantly, whereas during the other interventions neither HR nor contractility changed significantly (data not shown). In additional experiments in isolated hearts, we failed to find that the alterations in mechanical performance after combined application of the CGRP-R1 and AM receptor antagonists differed significantly from those evoked by simple flow reduction to corresponding values (n = 4) (data not shown).

In both control and LPS groups, BQ-123 and phosphoramidon were equipotent. This result indicates that coronary constriction was mediated by conversion of pulmonary big ET-1 rather than by direct action of the mature peptide.

Control Experiments in Isolated Hearts

In Figure 3, we depict the effects of exogenous vasoactive mediators on CF.

Coronary infusion of ET-1 at a concentration of 5 pM significantly decreased CF to 84 ± 3% of baseline value; an identical concentration of big ET-1 decreased CF to 86 ± 2%. ET_A receptor blockade by BQ-123 inhibited the effects of both ET-1 and big ET-1 completely, whereas ECE inhibition by phosphoramidon prevented only the big ET-1-induced decrement in CF. ET_B blockade by A-192621 had no effect (data not shown).

AM at a concentration of 100 pM increased CF to 134 ± 4% of baseline rate.

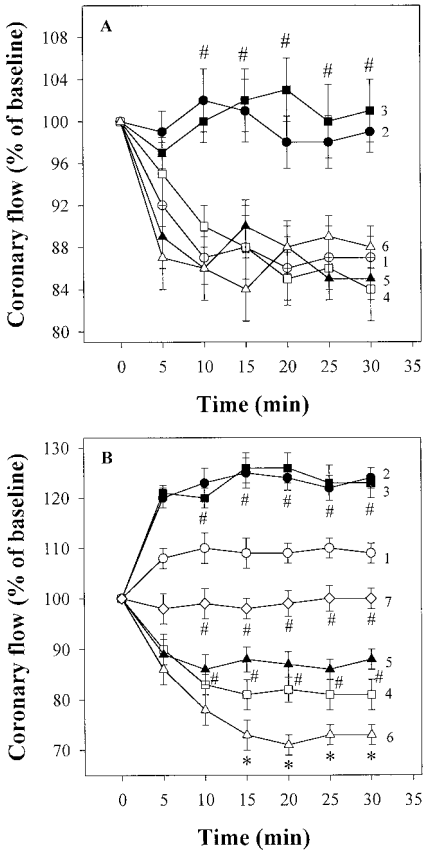

Figure 2. Coronary flow during serial perfusion of isolated hearts with (*A*) effluent of control lungs and (*B*) effluent of lungs pretreated with lipopolysaccharide (*LPS*; 50 μg/mL) over 2 hrs. *A*, curve 1 is control lungs; 2, the endothelin $(ET)_A$-selective antagonist BQ-123 (2 μM); 3, the ET-converting enzyme (*ECE*) inhibitor phosphoramidon (50 μM); 4, the calcitonin gene-related peptide type-1 receptor (*CGRP-R1*) antagonist hCGRP(8-37) (2 μM); 5, the adrenomedullin (*AM*) receptor antagonist hAM(22-52) (200 nM); and 6, hCGRP(8-37) plus hAM(22-52). *B*, curve 1 is LPS lungs; 2, the ET_A-selective antagonist BQ-123 (2 μM); 3, the ECE inhibitor phosphoramidon (50 μM); 4, the CGRP-R1 antagonist hCGRP(8-37) (2 μM); 5, the AM receptor antagonist hAM(22-52) (200 nM); 6, hCGRP(8-37) plus hAM(22-52); and 7, hCGRP(8-37) plus hAM(22-52) plus BQ-123. Six experiments were performed for each group. Switch from separate to serial perfusion was performed at time zero (baseline). Data are given as percentage of baseline coronary flow. #$p < .05$, vs. untreated control and untreated LPS; *$p < .05$, single application of hCGRP(8-37) or hAM(22-52) vs. hCGRP(8-37) plus hAM(22-52).

Both the CGRP-R1 antagonist hCGRP(8-37) and the AM receptor antagonist hAM(22-52) blocked this response partially, to 110 ± 3% and to 119 ± 2%, respectively. Combined application of these drugs completely suppressed the AM-induced elevation of CF.

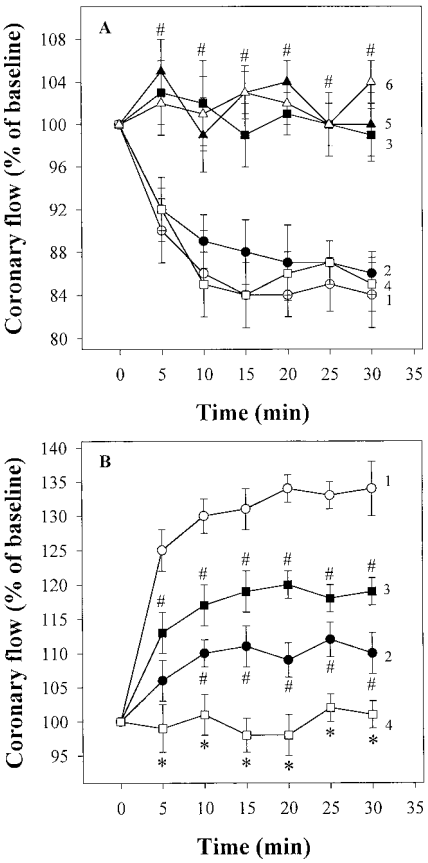

Figure 3. Coronary flow in isolated hearts in the presence of exogenous endothelin (*ET*)-1, big ET-1, and adrenomedullin (*AM*). Data are given as percentage of baseline coronary flow; n = 6 for each group. *A*, curve 1 is ET-1 (5 pM); 2, big ET-1 (5 pM); 3, ET-1 plus ET_A antagonist BQ-123 (2 μM); 4, ET-1 plus ET-converting enzyme inhibitor phosphoramidon (50 μM); 5, big ET-1 plus BQ-123 (2 μM); and 6, big ET-1 plus phosphoramidon (50 μM). *B*, Curve 1 is AM (100 pM); 2, AM plus calcitonin gene-related peptide type-1 receptor antagonist hCGRP(8-37) (2 μM); 3, AM plus AM receptor antagonist hAM(22-52) (200 nM); and 4, AM plus hCGRP(8-37) plus hAM(22-52). #$p < .05$, vs. untreated application of substances; *$p < .05$, single application of hCGRP(8-37) or hAM(22-52) vs. hCGRP(8-37) plus hAM(22-52).

Determination of ET and AM in Pulmonary Venous Effluent

Table 1 displays the concentrations detected for the different sampling periods during serial perfusion.

Big ET-1 and ET-1. In controls, pulmonary venous concentrations of big ET-1 and ET-1 during serial perfusion ranged between 4.3 ± 0.2 and 4.6 ± 0.3 pM and between 0.089 ± 0.007 and 0.102 ± 0.006 pM, respectively. LPS treatment significantly increased big ET-1 (approximately threefold) and ET-1 (twofold). In both groups, values differed only slightly at the various time intervals.

Adrenomedullin. At the various time intervals during serial perfusion, control lungs released between 9.6 ± 0.8 and 10.2 ± 0.7 pM into pulmonary venous effluent. After LPS treatment, these values incremented approximately five-fold and were measured between 48.5 ± 2.7 and 51.2 ± 3.1 pM.

DISCUSSION

We investigated the effect of pulmonary mediators on coronary vascular tone under conditions of experimental ARDS. We have recently shown pulmonary ETs to act—via ET_A receptors—as remote coronary vasoconstrictors (8, 9, 10). In the present study, we confirmed these findings in rats and further established that the basal pulmonary vasoconstrictor impact—exerted by big ET-1—transforms into a vasodilator effect after pulmonary LPS challenge. This vasodilation is primarily caused by elevated pulmonary AM as we were able to demonstrate by the fact that combined blockade of the two AM-binding receptors prevented a decrease in coronary tone. Correspondingly, we measured increased AM concentrations in the pulmonary effluent of LPS-treated lungs.

The AM in pulmonary effluent most likely originates from pulmonary vascular endothelial and smooth muscle cells. This conclusion is based on the fact that these cell types recently have been identified as the main source of the peptide (31, 32). In addition, enhanced expression of AM mRNA as well as elevated peptide synthesis has been reported *in vivo* (12), as well as after LPS challenge in cultured endothelial and vascular smooth muscle cells (31, 32).

With regard to the receptors involved in the coronary action of AM, our findings indicate contribution of both the CGRP-R1 and the AM receptor. To our knowledge, hAM(22-52) has not yet been tested in isolated rat hearts, but there are several reports in good concurrence with our findings. For example, Entzeroth et al. (33) reported significant attenuation, but not complete blockade, by hCGRP(8-37) of the AM-evoked increase in CF in isolated rat hearts. Sheykhzade et al. (23) found a significant population of CGRP-R1 in intramural coronary arteries of rats; however, they did not examine the potential role of AM receptors. In the rat aorta, the existence and functional rele-

Table 1. Pulmonary venous concentrations of big endothelin (ET)-1, ET-1, and adrenomedullin (AM) measured during serial perfusion

Time (mins)	Big ET-1 (pM)		ET-1 (pM)		AM (pM)	
	Control	LPS	Control	LPS	Control	LPS
0–10	4.3 ± 0.2	12.6 ± 0.8[a]	0.092 ± 0.005	0.172 ± 0.015[a]	10.2 ± 0.7	48.5 ± 2.7[a]
11–20	4.6 ± 0.3	13.3 ± 0.7[a]	0.102 ± 0.006	0.192 ± 0.017[a]	9.6 ± 0.8	50.2 ± 3.5[a]
21–30	4.5 ± 0.2	13.1 ± 0.6[a]	0.089 ± 0.007	0.206 ± 0.015[a]	9.9 ± 0.4	51.2 ± 3.1[a]

LPS, lipopolysaccharide.
[a] $p < .05$ vs. control.

vance of both receptors—the CGRP-R1 and the AM receptor—have been demonstrated recently (26).

In the isolated rat heart, Szokodi et al. (34) found inotropic effects at AM concentrations as low as 30 pM. Moreover, these authors documented vasodilatory effects at AM concentrations as low as 300 pM, although they used a model with marked coronary predilation due to limited perfusion rate. Considering these differences in experimental setup, the data indicate effectiveness of AM at concentrations that are comparable to those measured in pulmonary effluent in our LPS group (~50 pM) and to those used for exogenous application in isolated hearts (100 pM).

Parallel to the up-regulation of AM, expression of prepro-ET-1 mRNA and synthesis of big ET-1 and ET-1 are increased in lung tissue (11, 27). We were able to confirm these findings by measuring elevated concentrations of big ET-1 and ET-1 in pulmonary venous effluent of LPS-stimulated lungs. On inhibition of AM-induced coronary vasodilation, we unmasked a coronary vasoconstrictor effect that was significantly stronger than that observed in control preparations and that evoked significant contractile depression. Conversely, blockade of ET_A receptors or inhibition of ECE markedly augmented coronary vasodilation in comparison with that observed in untreated LPS preparations. Because coronary flow response in LPS preparations was completely reversed to preserial baseline values by use of the triple combination of ET and AM blockers (Fig. 2B), we can reasonably argue that the documented effects primarily reflect the opposite actions of elevated endogenous pulmonary AM and pulmonary big ET-1.

The effectiveness of BQ-123 to completely prevent coronary response to exogenous big ET-1 and ET-1 as well as during serial perfusion emphasizes the prevailing contribution of ET_A receptors to ET effects in the low picomolar range. Although involvement of smooth muscle ET_B receptors in coronary responses also has been reported, relevant ET_B-mediated effects usually are observed at markedly higher (i.e., low nanomolar) concentrations (35, 36).

For both ET-1 and AM, significant effects on cardiac inotropy have been reported (37–39). However, we were able to detect specific inotropic effects neither for big ET-1/ET-1 nor for AM. Other experiments, based on isolated hearts, also have failed to demonstrate those effects for ET-1 (40). With respect to AM, the findings are even more controversial because positive (38), negative (39), and indifferent effects (41) on inotropy have been disclosed. Thus, the issue of AM and inotropy remains to be completely unraveled.

For the *in vivo* situation as well as for future therapeutic strategies, we infer from our model that both the vasoconstrictor system (big ET-1 and ET-1) and the counteracting mediator (AM) are upregulated in pulmonary vasculature and may achieve pathophysiologically relevant concentrations in pulmonary outflow. It also seems important that endogenous pulmonary AM potentially may neutralize the deleterious remote effects of endogenous pulmonary ETs. From our model, we cannot precisely predict the magnitude of coronary changes under distinct *in vivo* conditions. It recently has been demonstrated that plasma concentrations of AM increase dramatically (10- to 40-fold) in patients with sepsis and systemic inflammatory response syndrome, with values detected as high as 50–200 pM (42–44). Moreover, AM plasma concentrations correlate with systemic and pulmonary vascular resistance (43) as well as with circulating concentrations of tumor necrosis factor-α (44) in these patients. These facts may accentuate the potential clinical relevance of our findings. On the other hand, several factors including coronary endothelial dysfunction (8, 9) and enhancement of pulmonary release of ETs by activated neutrophils (45) may tip the balance between AM and ET-1 toward coronary constriction. Considering the remote effects of pulmonary mediators, our study underlines the potential usefulness of ET-1 antagonists as a therapeutic tool in ARDS, because ET-1 blockade potentiates AM-induced coronary dilation. Until now, there have been no clinical data regarding administration of ET antagonists in human ARDS, sepsis, or systemic inflammatory response syndrome (46). Compelling evidence, however, has been gathered from different animal models indicating that blockade of ET_A receptors or mixed ET antagonism (ET_A and ET_B) improves systemic and pulmonary hemodynamics (46), decreases retention of leukocytes in the lung (47), and alleviates nitric oxide-mediated lung injury (48).

We conclude that in experimental ARDS, elevated pulmonary AM—by occupation of coronary CGRP type-1 receptors and AM receptors—outweighs the coronary vasoconstrictor impact of pulmonary big ET-1 exerted via ET_A receptors after conversion to mature ET-1. The consequence is prevention of flow-related deterioration of myocardial performance.

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