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Brief adrenomedullin inhalation leads to sustained reduction of pulmonary artery pressure

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Brief adrenomedullin inhalation leads to sustained reduction of pulmonary artery pressure. K. von der Hardt, M.A. Kandler, M. Chada, A. Cubra, E. Schoof, K. Amann, W. Rascher, J.Dötsch. ©ERS Journals Ltd 2004.

ABSTRACT: The effect of aerosolised adrenomedullin (ADM), a potent vasodilator peptide, on pulmonary artery pressure was studied for 24 h in a surfactant-depleted piglet model.

Animals received either aerosolised ADM (50 ng·kg^{-1}·min^{-1}, ADM, n=6), or aerosolised normal saline solution (control, n=6). Aerosol therapy was performed for a 2 h treatment period followed by a 22 h observation period. Ventilator settings were adapted to keep arterial oxygen tension and carbon dioxide arterial tension between 13.3–14.6 kPa and 4.9–5.7 kPa, respectively.

Aerosolised ADM reduced mean pulmonary artery pressure (MPAP) compared with the control group (end-point median 24 h after therapy start: ΔMPAP -14.0 *versus* -8.0 mmHg; 23.5 h after therapy start). After therapy start, mean systemic arterial pressure (MAP) was not significantly different between the groups (end-point median: MAP ADM 70 (61/74) *versus* control 72 (54/81) mmHg). Endothelin-1, a potent pulmonary vasoconstrictor, is regulated by ADM *via* cAMP. Twenty two hours after inhalation of aerosolised ADM, endothelin-1 mRNA in lung tissue and endothelin-1 protein expression in pulmonary arteries was reduced compared with controls (median semi-quantitative immunhistochemical score: ADM 0.21, control 0.76).

Aerosolised adrenomedullin significantly reduced mean pulmonary artery pressure independently of arterial oxygen tension.

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Pulmonary hypertension is often associated with an imbalance of vasoconstrictor and vasodilator peptides [1]. Endothelin-1 (ET-1) is the most potent constrictor and has been implicated in the pathogenesis of pulmonary hypertension [2]. Adrenomedullin (ADM), which consists of 52 amino acids, is actively produced and secreted by various cell types, particularly the vascular endothelial and smooth muscle cells [3], and has potent vasodilator properties. Transgenic mice overexpressing ADM in their vasculature have been shown to be resistant to lipopolysaccharide (LPS)-induced shock. LPS caused smaller drops in blood pressure and less severe organ damage than in the wild-type, despite lower basal blood pressure [4]. Moreover, ADM overexpression reduced reperfusion injury and superoxide production [5]. ADM$^{-/-}$ knock-out mice showed lethal cardiovascular pathology *e.g.* extreme hydrops foetalis [6], mice with ADM$^{+/-}$ mutation exhibited elevated blood pressures with diminished nitric oxide production and increased oxidative stress [7, 8], suggesting a crucial role for ADM in the regulation of the cardiovascular system, particularly endothelial cell function. ADM plays an important role in vascular morphogenesis and the regulation of blood pressure by stimulating nitric oxide production [7].

Intravenous infusion of ADM in patients with pulmonary hypertension significantly reduced pulmonary vascular resistance. However, systemic vascular resistance was simultaneously decreased [9]. Similar effects were seen with the application of ADM to the pulmonary artery [10]. To increase the pulmonary selectivity of ADM-induced vasodilation, the current authors examined the effect of inhaled ADM on pulmonary artery pressure and systemic blood pressure, investigating interval doses 5.0–100 ng·kg^{-1}·min^{-1}. In this study, application of aerosolised ADM did not lead to a reduction of systemic arterial pressure and the reduction of pulmonary artery pressure by aerosolised ADM did not subside within 3 h after the discontinuation of ADM inhalation [11]. Moreover, *post mortem* analysis of lung tissue showed a reduction of interleukin (IL)-1β and transforming growth factor-β gene expression and IL-1β protein concentration [12]. Since a significant improvement of oxygenation was seen after aerosolised ADM, the proportional contribution of oxygenation to the pulmonary vasodilator effect of ADM could not be defined. Therefore, in the present study, animals were kept at constant arterial oxygen tension (P_aO_2) after the inhalation of ADM (50 ng·kg^{-1}·min^{-1}) in both the ADM and control groups.

Since ADM increases intracellular cyclic AMP concentration [13, 14], which is itself a vasorelaxant [15], but also suppresses ET-1 synthesis [16], the current study assessed ET-1 mRNA gene and protein expression in lung tissue.

The first objective of the present study was to examine whether short-term inhalation of ADM for 2 h would lead to

For editorial comments see page 518.

a sustained reduction of pulmonary artery pressure for 24 h. The second objective was to investigate whether the effect of ADM on pulmonary vasodilation is maintained when constant P_{a,O_2} and constant carbon dioxide arterial tension (P_{a,CO_2}) are provided.

Material and methods

Study animals

The study was approved by the Animal Care Committee of the University of Erlangen and the government of Mittelfranken, Germany, and performed according to the European community guidelines for the use of experimental animals.

Twelve piglets with a body weight of 3.3–4.1 kg were included in the study. After a venous catheter had been placed into an ear vein, anaesthesia was induced with midazolam (1 mg·kg^{-1}), fentanyl (2.5 μg·kg^{-1}) and ketamine (5 mg·kg^{-1}), followed by continuous infusion of midazolam (1.5 mg·kg^{-1}·h^{-1}), fentanyl (0.01 mg·kg^{-1}·h^{-1}) and ketamine (15 mg·kg^{-1}·h^{-1}). After tracheotomy, paralysis was induced with vecuronium 0.2 mg·kg^{-1} *i.v.* and maintained with vecuronium 0.2 mg·kg^{-1}·h^{-1}. A central venous catheter (4.5 F; Cook®, Mönchengladbach, Germany) was placed into the right jugular vein and a pulmonary catheter (4 F; Arrow®, Erding, Germany) was placed into the pulmonary artery. After preparation of the left femoral artery, an arterial catheter (20 G; Arrow®) and a sensor for online blood gas monitoring (Paratrend 7®; Philips®, Böblingen, Germany) were inserted for online registration of blood gases. The piglets received a transcutaneous urinary catheter (Cystofix minipäd®; Braun, Melsungen, Germany). Dynamic compliance and resistance were recorded with a hot wire anemometer (MIM® GmbH, Krugzell, Germany), computed with the neonatal respiration monitoring Florian® NRM-200 (MIM®). Heart rate, central venous, pulmonary artery and arterial pressure were continuously recorded (CMS 2001; Philips®). Cardiac index (CI) was measured by thermodilution method before lavage and at the end of the observation period. Systemic vascular resistance index (SVRI) was computed as follows:

$$\mathrm{SVRI} = 79.9 \times (\mathrm{MAPCVP})/\mathrm{CI}\ (\mathrm{dyn \cdot s^{1} \cdot cm^{5} \cdot m^{2}}) \quad (1)$$

Where MAP is mean arterial pressure and CVP is central venous pressure. Pulmonary vascular resistance index (PVRI) was computed as follows:

$$\mathrm{PVRI} = 79.9 \times (\mathrm{MPAPPCWP})/\mathrm{CI}\ (\mathrm{dyn \cdot s^{1} \cdot cm^{5} \cdot m^{2}}) \quad (2)$$

Where MPAP is mean pulmonary arterial pressure and PCWP is pulmonary capillary wedge pressure. Arterial blood gas analysis was performed at 30 min intervals (ABL 330, Radiometer, Copenhagen, Denmark). Intermittent mandatory ventilation was performed with a neonatal respirator (Infant Star 950; Mallinckrodt^{TM}, Hennef, Germany). During instrumentation, induction of lung injury and ADM inhalation, breath rate was 50 breaths·min^{-1}, peak inspiratory pressure 32 cm H_2O, positive end expiratory pressure 8 cm H_2O and the inspiratory fractional oxygen concentration (F_{I,O_2}) 1.0. Respiratory gas was humidified and the temperature was adjusted to 39°C (MR 700; Fischer & Paykel®, Welzheim, Germany). During instrumentation and for the duration of the experiment, animals were in supine position. Lung injury with pulmonary hypertension was induced by surfactant-depletion (repeated saline lung lavage). NaCl 0.9% (30 mL·kg^{-1}; 39°C) was instilled into the endotracheal tube, reaching the left and right side of the lung depending on the position of the animal (alternating left and right) [17]. Piglets were suctioned after each lavage. When the lung injury was considered stable, defined as P_{a,O_2} remaining consistently <9.3 kPa for 60 min, the piglets were included in the study. If inclusion criteria failed, repeated lung lavages were performed until the criteria were met.

Methods

After attaining inclusion criteria, the animals were randomly assigned to two different therapy groups (ADM and control). In all animals, respiratory support was maintained constant at identical respiratory settings during aerosol treatment. ADM (50 ng·kg^{-1}min^{-1}, Bachem®, Heidelberg, Germany) was aerosolised with a jet aerosol device (AerProbe®; Trudell®, London, Canada) in saline solution (4 mL·h^{-1}) over 2 h. Piglets in the control group received aerosolised saline solution at equal volume. After the aerosol treatment, F_{I,O_2} and respiratory rate were continuously adjusted to keep P_{a,O_2} and P_{a,CO_2} within normal ranges for 22 h (P_{a,O_2} 1.3–1.9 kPa and P_{a,CO_2} 0.7–0.8 kPa) to avoid oxygen induced vasodilation or CO_2 induced vasoconstriction. After this observation period (24 h after the establishment of lung injury), the animals were sacrificed by *i.v.* injection of 50 mg·kg^{-1} methohexital and 20 mL potassium chloride 7.46%. Lungs and heart were removed together. Tissue samples were taken from the right lung. mRNA extraction and reverse transcription was performed with guanidine-thiocyanate acid phenol (RNAzol; WAK Chemie®, Medical GmbH, Bad Homburg, Germany). RNA (1 mL) was reverse transcribed in a volume of 20 μL at 39°C for 60 min (all chemicals were obtained from Boehringer® Mannheim, Germany). Efficiency and reliability of TaqMan real time PCR have been shown earlier [18]. The use of TaqMan real time PCR in this animal model was published recently [11, 12]. mRNA expression of ET-1 and IL-1β mRNA was normalised to hypoxanthine-guanine-phosphoribosyl-transferase (HPRT) as a housekeeping gene. Primers and TaqMan probes were elected for the porcine model (table 1).

For immunohistology, 3 μm paraffin sections were cut, dewaxed and incubated in 3% H_2O_2 (20 min, room temperature) and subsequently with pronase (10 min, room temperature). Staining was then performed using an unconjugated ET-1 antibody (polyclonal rabbit; BioTrend Co., Cologne, Germany, 1:300, 1 h at 37°C) and a second antibody (goat antirabbit, Vektor *via* Alexis Deutschland Co. Grünberg, Germany, 1:200, 30 min, room temperature). The samples were then rinsed in Tris-buffered saline (pH 7.6) and submitted to the avidin-biotin system with tyramid enhancement (TSA-Biotin System; NEN Life Science, Cologne, Germany). Each incubation step was followed by a thorough

Table 1. – Primers and TaqMan probes

Endothelin-1
Forward 5′- CTCCTGCTCTTCCCTGATGG -3′
Reverse 5′- TGGCACACTGGCATCTATCC -3′
TaqMan probe 5′(FAM)- TTCTGCCACCTGGACATCATT-TGGG -(TAMRA)3′
Interleukin-1β
Forward 5′ GGTTTCTGAAGCAGCCATGG -3′
Reverse 5′ GATTTGCAGCTGGATGCTCC -3′
TaqMan probe 5′ (FAM)- AAAGAGATGAAGTGCTGCAC-CCAAAACCTG -(TAMRA)3′
Hypoxanthine-guanine-phosphoribosyl-transferase
Forward 5′- CGGCTCCGTTATGGCG -3′
Reverse 5′- GGTCATAACCTGGTTCGTCATCA -3′
TaqMan probe 5′(FAM)- CGCAGCCCCAGCGTCGTGATTA--(TAMRA)3′

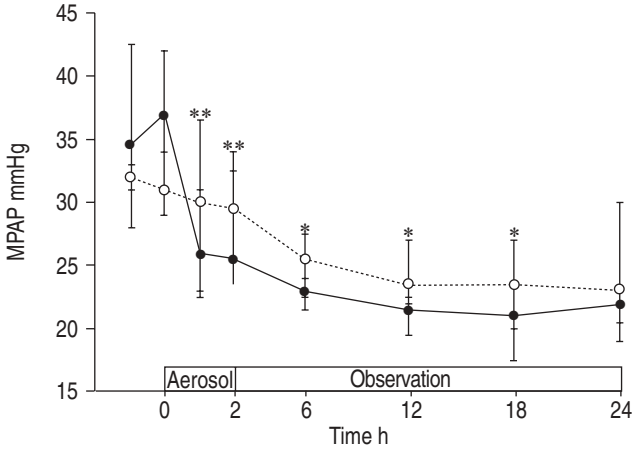

Fig. 1. – Mean pulmonary arterial pressure (MPAP) obtained after induction of lung injury, at start of treatment (T0), during therapy with aerosolised adrenomedullin (ADM; ●) and with normal saline (control group; ○) and during the post-treatment observation time in surfactant-depleted neonatal piglets. Data are presented as median±25th/75th percentile. *: $p<0.05$; **: $p<0.01$ MPAP reduction from baseline (T0), ADM *versus* control.

double rinse in Tris-buffered saline. AEC (Dako GmbH, Hamburg, Germany) served as a chromogen. Subsequently, sections were counterstained with Mayers haemalum (Merck Co., Darmstadt, Germany) and examined by light microscopy. Negative controls were performed by omitting the primary antibody. Staining of all sections was performed in the same run to reduce inter-run variations in staining intensity. Endothelial ET-1 protein expression was evaluated at high magnification in 3–21 pulmonary arteries per animal (mean 8.23±4.15) using a semi-quantitative scoring system (0=no, 1=mild, 2=moderate, 3=strong ET-1 expression).

Data analysis

The data was recorded before and after the induction of lung injury (baseline; 0), during therapy and during the post-treatment observation time. A p-value of <0.05 was considered significant. Values are expressed as median (25th percentile/75th percentile). The Mann-Whitney U-test was used for comparison between groups.

Results

Mean pulmonary artery pressure

Induction of lung injury by bronchoalveolar lavage increased MPAP from 15.5 (15.0/17.0) to 37.0 (31.0/42.0) mmHg (median (25 P/75 P)) in the ADM group and from 17.0 (14.5/20.5) to 31.0 (29.0/34.0) mmHg in the control group. Values were not significantly different between the groups before treatment. Aerosolised ADM reduced MPAP significantly compared with the control group (fig. 1 and 2). In addition, the decline of MPAP was significantly faster in the

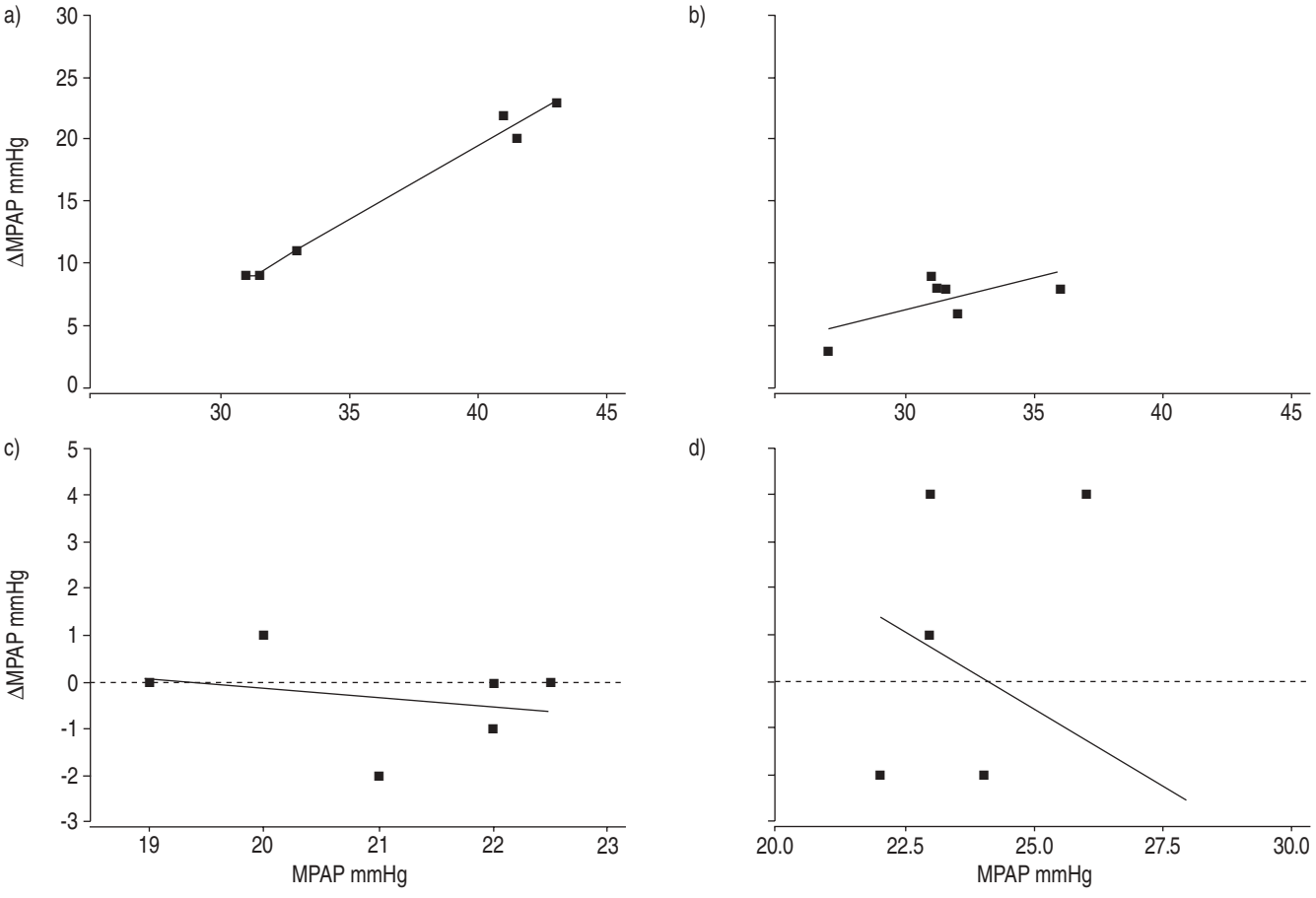

Fig. 2. – Linear correlation of change in mean pulmonary arterial pressure (ΔMPAP) *versus* MPAP during the first 12 h in a) adrenomedullin (ADM) and b) control groups and during the second 12 h in c) ADM and d) control groups.

ADM than in the control group. The reduction in MPAP continued until 10 h after ADM aerosol treatment. Reduction in MPAP was significantly different from control up to 23.5 h after the start of the intervention.

Mean arterial pressure

During ADM aerosol inhalation and in the following 22 h there was no significant difference in MAP between the ADM and the control groups (fig. 3). However, there was a significant decrease in MAP in the ADM group, which was not found in the control group (table 2).

Arterial oxygen and carbon dioxide tension

There were no differences in P_{a,O_2} and P_{a,CO_2} between the ADM and control groups during the observation period (fig. 4 and 5) due to adaptation of respirator settings. The difference in $P_{a,O_2}/F_{I,O_2}$ (Horowitz index) during ADM inhalation did not reach significance (fig. 6).

Mixed venous oxygen tension

During ADM aerosol application, mixed venous oxygen tension (P_{v,O_2}) rose significantly ($p<0.01$) compared with the control group ($\Delta P_{v,O_2}$ $p<0.05$ at 1, 12, and 18 h, $p<0.01$ at 2, 6, and 24 h after the start of treatment). P_{v,O_2} was significantly higher during ADM aerosol inhalation and in the observation period than in controls after treatment start (table 2).

Respiratory resistance and dynamic compliance

There were no differences in respiratory resistance or dynamic compliance between the ADM and control group during treatment or during the observation period (table 2).

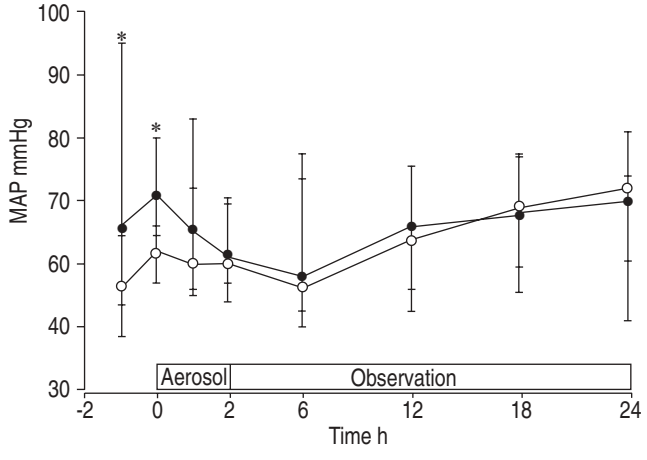

Fig. 3. – Mean arterial pressure (MAP) obtained after induction of lung injury, at the start of treatment (T0), during therapy with aerosolised adrenomedullin (ADM; ●) and with normal saline (control group; ○) and during the post-treatment observation time in surfactant-depleted neonatal piglets. Data are presented as median±25th/75th percentile. *: $p<0.05$ MAP reduction from baseline (T0), ADM *versus* control.

Cardiac index, pulmonary capillary wedge pressure, vascular resistance indices

There were no differences in CI (ADM 0.72 (0.63/0.82), control 0.73 (0.58/1.01) $L \cdot min^{-1} \cdot m^{-2}$), PCWP (ADM 7.63 (6.5/10.0), control 8.0 (3.5/8.0) mmHg), SVRI (ADM 7201 (6121/8356), control 6410 (6024/9005) $dyn \cdot s^{-1} \cdot cm^{-5} \cdot m^{-2}$) or PVRI (ADM 1712 (1030/2261), control 2014 (1344/2338) $dyn \cdot s^{-1} \cdot cm^{-5} \cdot m^{-2}$) between the groups 24 h after treatment with aerosolised ADM.

IL-1β gene expression

IL-1β/HPRT mRNA expression in lung tissue 22 h after inhalation of aerosolised ADM was 16.6 (8.8/36.5) relative units (RU) (median (25th percentile/75th percentile)) for the ADM group and 23.5 (14.2/49.4) RU for the control group ($p=0.07$).

Endothelin-1 gene expression

ET-1/HPRT mRNA expression in lung tissue 22 h after inhalation of aerosolised ADM was reduced compared with controls (ADM: 23.5±1.94 RU (mean±SEM), control: 36.1±4.42 RU, $p<0.05$ (fig. 7), ADM: 21.1 (14.4/28.5) RU (median (25th percentile/75th percentile)), control: 27.5 (14.3/49.2) RU, $p=0.09$).

Endothelin-1 immunhistology in pulmonary arteries

ET-1 protein expression was significantly reduced in endothelial cells in pulmonary arteries of ADM treated animals compared with controls. Semi-quantitative ET-1 score was 0.21 (0.03/0.28) in ADM treated and 0.76 (0.50/1.21) in control animals, $p<0.01$. In the arterial media, there was hardly any ET-1 protein expression (fig. 8).

Discussion

Pulmonary hypertension is a typical feature of acute respiratory distress syndrome (ARDS) and is caused mainly by pulmonary vasoconstriction due to hypoxia and the release of vasoconstrictive inflammatory mediators. ARDS associated with pulmonary hypertension is a typical constellation encountered in a clinical setting, and is associated with high mortality. Since proning of ARDS patients may improve ventilation and oxygenation [19, 20], animals in this study were kept in a supine position to minimise uncontrolled effects.

The beneficial role of ADM with regard to cardiovascular protection has previously been intensively studied [5, 8, 9, 21]. In the present study, a 2 h period of ADM inhalation significantly reduced pulmonary artery blood pressure. The attenuation was twice as high as in the control group. In contrast to experiments using inhaled nitric oxide or iloprost, the reduction of pulmonary artery pressure persisted for hours after the withdrawal of ADM. To the authors' knowledge, none of the standard inhalative therapies for pulmonary hypertension, such as iloprost or nitric oxide, shows a comparable long-term effect [22, 23]. Therefore, treatment with aerosolised ADM may be an option to reduce pulmonary arterial pressure persistently. In contrast, systemic administration of ADM provides only a short-term reduction of pulmonary artery resistance, which subsides a few minutes after ADM

Table 2. – Delta mean pulmonary arterial pressure (ΔMPAP), mean arterial pressure (MAP), arterial oxygen tension (P_{a,O_2}), arterial carbon dioxide tension (P_{a,CO_2}), mixed venous oxygen tension (P_{v,O_2}), Horowitz index ($P_{a,O_2}/F_{i,O_2}$), dynamic compliance, respiratory resistance, central venous pressure (CVP) and heart rate in adrenomedullin (ADM) and control groups

	Baseline	2 h treatment	Time after baseline h			
			6	12	18	24
ΔMPAP mmHg						
ADM		-8.5*	-15.0*	-15.5**	-13.5*	-13.5
%		-23.1	-40.1	-41.1	-36.5	-37.1
25P		-14.0	-19.0	-22.5	-24.5	-23.0
75P		-4.5	-7.5	-9.0	-10.0	-8.5
Control		-2.0	-6.5	-8.0	-8.0	-8.0
%		-6.9	-20.7	-24.0	-25.4	-25.8
25P		-6.0	-10.5	-8.5	-11.0	-8.5
75P		-0.5	-1.5	-4.5	-4.5	-4.0
P_{a,O_2} mmHg						
ADM	53	125	112	112	106	97
25P	48	74	112	104	99	75
75P	60	147	136	123	134	123
Control	56	92	113	119	116	107
25P	44	59	87	101	98	84
75P	64	116	125	130	124	129
ΔMAP mmHg						
ADM		-8.5*	-7.5	-9.5	-8.0*	-4.5
%		-11.6	-10.5	-13.3	-11.3	-5.8
25P		-15.0	-18.5	-14.0	-11.0	-10.0
75P		-3.0	-1.5	2.0	1.0	3.5
Control		-1.0	-2.0	5.0	7.5	11.5
%		-1.7	-3.5	7.9	12.1	18.7
25P		-4.0	-10.5	-6.0	-2.0	-8.0
75P		5.0	8.0	11.5	15.5	19.0
P_{a,Co_2} mmHg						
ADM	52.2	36.0	38.9	39.0	39.1	44.2
25P	39.3	34.8	35.3	36.2	38.3	38.1
75P	60.7	38.7	41.6	40.6	40.4	51.1
Control	53.6	40.3	39.0	39.3	38.9	37.0
25P	49.2	36.4	35.4	32.0	35.7	34.9
75P	63.5	46.8	41.5	43.9	41.3	40.6
P_{v,O_2} mmHg						
ADM	35.7	43.1*	44.3**	42.6	41.1*	40.4**
25P	33.7	39.1	42.1	38.6	36.4	36.0
75P	38.0	46.3	47.2	46.3	47.2	45.0
Control	34.9	34.8	35.7	33.1	34.0	32.6
25P	30.4	29.2	28.2	28.1	29.7	27.1
75P	39.8	38.3	39.6	43.9	39.2	36.2
$P_{a,O_2}/F_{I,O_2}$						
ADM	53.4	168	210	222	144	130
25P	47.7	84.8	186	155	127	89.8
75P	59.9	217	441	256	226	208
Control	56.3	92.7	206	211	206	183
25P	44.5	58.7	102	135	125	104
75P	64.4	209	258	266	272	258
Dynamic compliance $mL \cdot cm^{-1}\ H_2O \cdot kg^{-1}$						
ADM	0.44	0.51	0.54	0.51	0.50	0.46
25P	0.40	0.42	0.48	0.46	0.46	0.39
75P	0.51	0.62	0.60	0.55	0.61	0.52
Control	0.42	0.44	0.47	0.50	0.46	0.48
25P	0.35	0.38	0.40	0.42	0.37	0.38
75P	0.44	0.47	0.49	0.56	0.52	0.56
Resistance cm $H_2O \cdot L^{-1} \cdot s^{-1}$						
ADM	121	138	142	115	112	114
25P	107	106	119	101	102	106
75P	127	164	148	119	126	137
Control	125	118	120	105	117	123
25P	119	110	108	96	102	96
75P	127	153	150	139	154	159
CVP mmHg						
ADM	5.0	5.0	4.0	4.0	4.0	4.0

(continued on next page)

Table 2. – (Continued)

	Baseline	2 h treatment	Time after baseline h			
			6	12	18	24
25P	4.0	4.0	3.5	3.5	3.5	3.5
75P	5.5	7.5	7.0	4.5	4.5	6.5
Control	4.0	4.0	5.0	3.0	4.0	3.0
25P	2.0	1.5	1.5	2.0	1.5	1.5
75P	9.0	6.0	6.5	4.0	4.0	4.5
Heart rate ·min^{-1}						
ADM	184	159	131	141	127	148
25P	119	125	113	114	120	108
75P	223	199	169	176	181	164
Control	159	169	141	157	157	180
25P	136	136	120	131	130	128
75P	208	188	189	191	182	186

Data are presented as median, per cent, 25th percentile (25P) and 75th percentile (75P). *: $p<0.05$; **: $p<0.01$. kPa=mmHg×0.133.

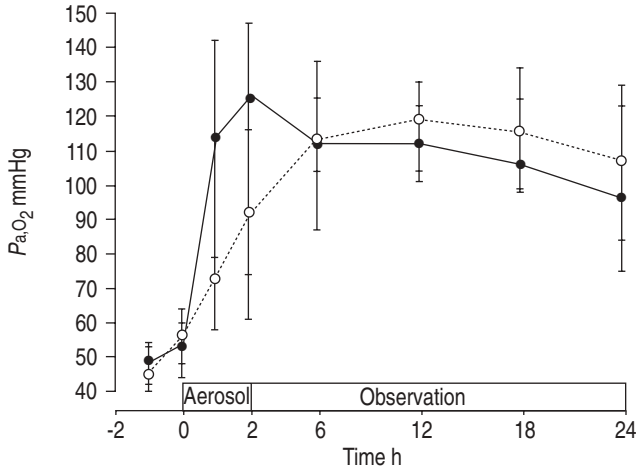

Fig. 4. – Arterial oxygen tension (P_{a,O_2}) obtained after induction of lung injury, at start of treatment (T0), during therapy with aerosolised adrenomedullin (●) and with normal saline (control group; ○) and during the post-treatment observation time in surfactant-depleted neonatal piglets. Data are presented as median±25th/75th percentile.

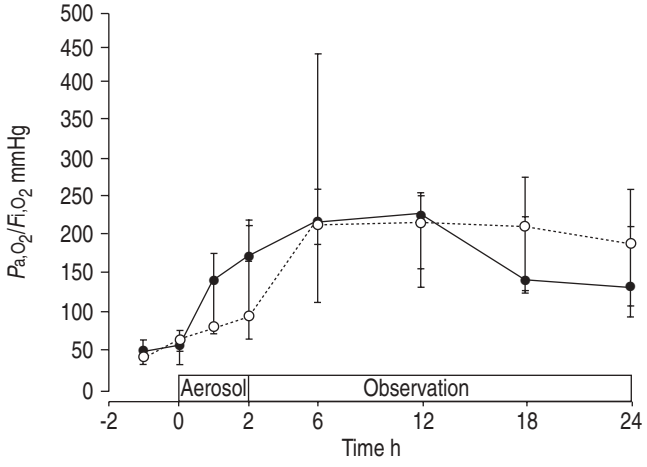

Fig. 6. – Arterial oxygen tension (P_{a,O_2})/inspiratory oxygen fraction (F_{I,O_2}) (Horowitz index) obtained after induction of lung injury, at start of treatment (T0), during therapy with aerosolised adrenomedullin (●) and with normal saline (control group; ○) and during the post-treatment observation time in surfactant-depleted neonatal piglets. Data are presented as median±25th/75th percentile.

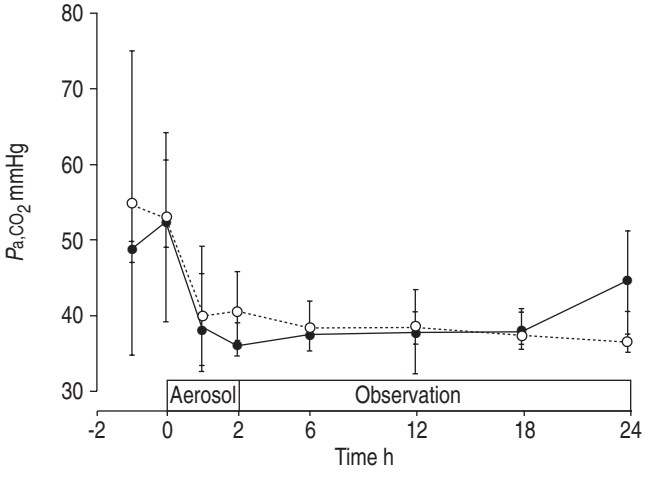

Fig. 5. – Arterial carbon dioxide (P_{a,CO_2}) obtained after induction of lung injury, at start of treatment (T0), during therapy with aerosolised adrenomedullin (●) and with normal saline (control group; ○) and during the post-treatment observation time in surfactant depleted neonatal piglets. Data are presented as median±25th/75th percentile.

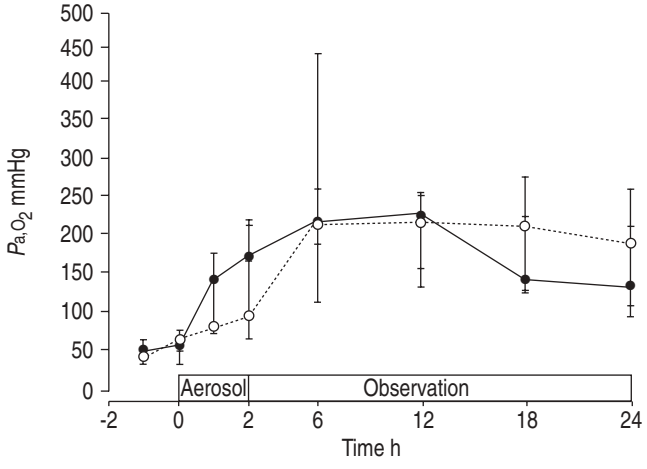

Fig. 7. – Endothelin-1/Hypoxanthine-guanine-phosphoribosyl-transferase (HPRT) mRNA expression in lung tissue of surfactant-depleted piglets *post mortem*, after therapy with aerosolised adrenomedullin (ADM) or with aerosolised normal saline (control group) during intermittent mandatory ventilation and an additional observation period of 22 h. Data are presented as relative units, median±25th/75th percentile. *: $p<0.05$ ADM *versus* control.

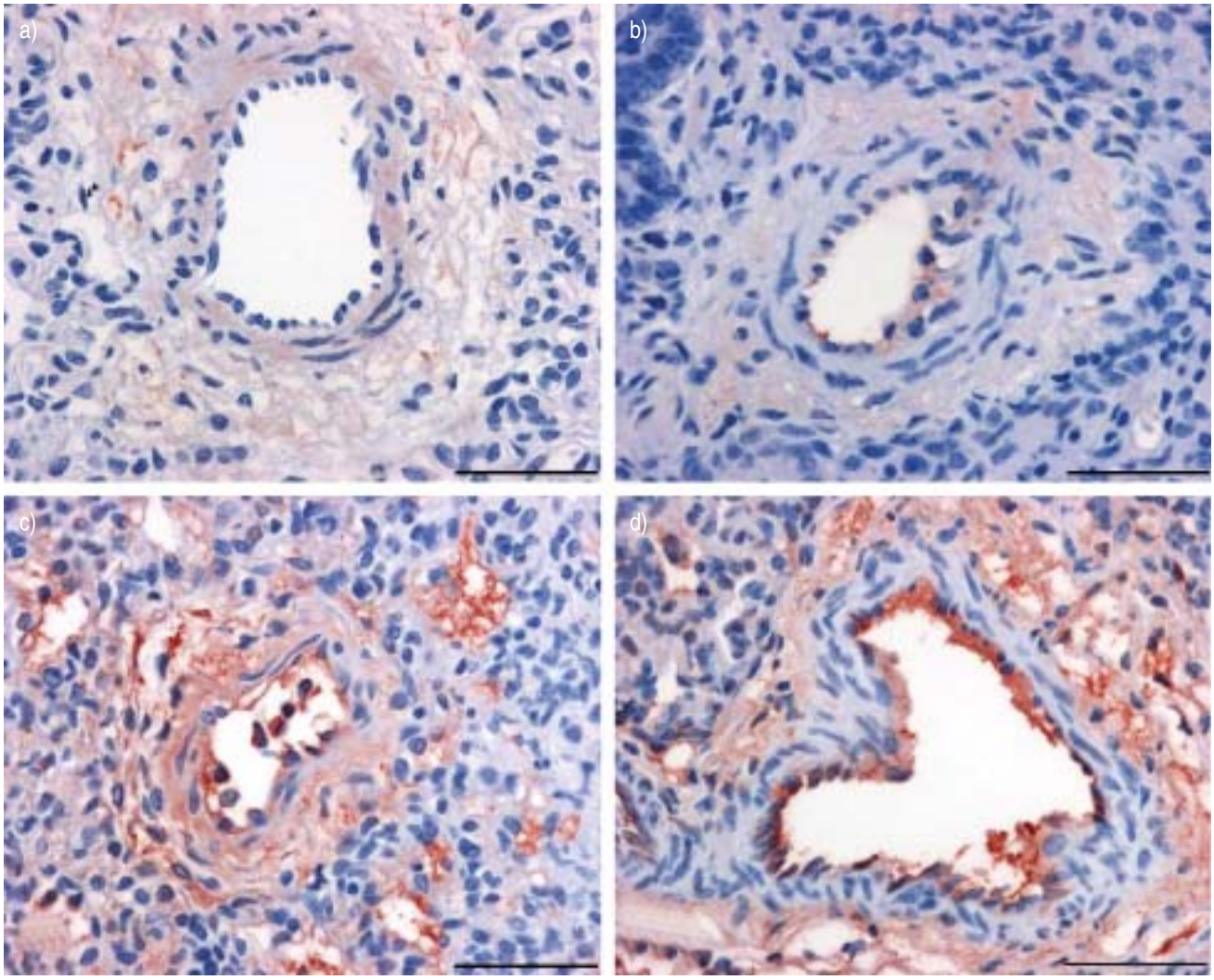

Fig. 8. – Endothelin-1 protein expression in pulmonary arteries of the anterior (a, c) and posterior (b, d) lower lobe of surfactant depleted piglets 22 h after a 2 h treatment period with aerosolised adrenomedullin (a, b) or with aerosolised normal saline (control) (c, d). Scale bar=50 μm.

withdrawal [9] and induces a considerable reduction in systemic arterial blood pressure. In a previous study, the vasodilation by aerosolised ADM was selective for pulmonary vessels without lowering systemic blood pressure [11]. In the present study, the significant decrease in MAP compared with control may be due to the significantly higher MAP at therapy start in the ADM group or may be caused by differences in ADM aerosol dosing (50 $ng \cdot kg^{-1} \cdot min^{-1}$ continuously for 2 h compared with a 30 min interval therapy in the previous study). As the absolute MAP values in the ADM and control group were identical, this finding is not of great importance. The exact mechanism of ADM-induced pulmonary vasodilation has not yet been completely resolved. One known mechanism of ADM vasodilation is the activation of endothelial NO synthases [24], however, the ADM effect is not mediated exclusively *via* NO [25]. The authors have previously reported in a pilot study of surfactant-depleted piglets, that the inhibition of NO synthases with L-NAME did not alter the pulmonary vasodilative effect of aerosolised ADM [11, 26]. Other mechanisms may therefore be of predominant importance. As previously demonstrated, oxygenation is improved during and after therapy with aerosolised ADM [11]. As improved oxygenation may itself lead to pulmonary vasodilation, the proportional contribution of improved P_{a,O_2} to pulmonary vasodilation could not be defined in the previous study. The authors therefore adjusted ventilator settings to maintain P_{a,O_2} and P_{a,CO_2} at constant physiological levels. This long-term model with identical P_{a,O_2} in the ADM and control group now clearly demonstrates that the pulmonary vasodilative effect of aerosolised ADM is not mediated *via* oxygen. A bias due to different inspiratory oxygen concentrations between the groups in favour of the ADM group can be excluded, as inspiratory oxygen concentration was not higher in the ADM group. As there was no significant difference in F_{I,O_2} or $P_{a,O_2}/F_{I,O_2}$ between the groups, the effect of ADM on oxygenation has to be regarded as marginal in this study. However, the improvement of P_{v,O_2} may indicate augmented oxygen transport to peripheral tissue, but oxygen transport was not investigated in this study. Dynamic compliance and respiratory resistance did not change significantly during the experiment and a bronchodilator response to ADM must remain speculative. Reduced ET-1 gene expression in the ADM group as seen in the present long-term study and also in the previous pilot study [11] demonstrates a possible ADM effect *via* reduction of the vasoconstrictive functional ADM antagonist ET-1. The potential importance of this mechanism is demonstrated by reduced ET-1 protein expression in pulmonary arteries of the ADM-treated group.

Within the respiratory tract, ET-1 is a potent vasoconstrictor

of pulmonary artery vessels and plays an important role in pulmonary hypertension. The reduction of ET-1 gene and protein expression following treatment with aerosolised ADM could be mediated by the suppressive effect of the ADM second messenger cAMP on ET-1 synthesis [13, 16]. Future studies on ET-1 antagonists may indicate whether this is the predominant mechanism. Reduction of ET-1 expression could contribute especially to the observed prolonged effect of aerosolised ADM on pulmonary artery pressure. cAMP is also a vasorelaxant and may contribute to the acute vasodilative effect of ADM. An acute vasodilative effect of ADM has been shown to be mediated, at least in part, by activation of nitric oxide synthases [27]. Alternatively, ADM peptide could accumulate in the alveolar space during inhalation, leading to a prolonged effect on the pulmonary vascular bed. As the biological half life of ADM is quite short (18 min) [21], this mechanism is rather unlikely. In addition, it may be speculated that the mechanism of ADM action is *via* an increased activity of prostaglandins or is exerted by activation of calcitonin gene-related peptide receptor [10]. The ADM effect may be increased by previous hypoxia, which has been shown to upregulate ADM receptor function [28]. ADM effects on pulmonary vascular resistance could not be recorded in the current study, the considerable side-effects of CI measurement by thermodilution and registration of pulmonary capillary wedge pressure in piglets with severe ARDS allowed measurement of these parameters only before and after the experiment. Possible effects on pulmonary vascular resistance may, therefore, not have been detected. Reduction of IL-1β mRNA expression in lung tissue almost reached significance. The lack of definitive significance may be due to the small number of piglets enrolled and the considerably long observation period of 22 h after the end of ADM application. How and where ADM or its mediators (*e.g.* cAMP or NO) reach the pulmonary artery system *via* the bronchiolo-alveolar system has not yet been defined and cannot be defined by the data of the current study. The study is limited by the use of an ARDS model of pulmonary hypertension, since the vasodilative effect of aerosolised ADM may be restricted to the model investigated. The effects of aerosolised ADM on pulmonary hypertension should be re-evaluated using another animal model of pulmonary hypertension. A direct comparison with inhaled NO or iloprost would be additionally helpful.

In conclusion, aerosolised adrenomedullin significantly reduces the mean pulmonary artery pressure independently of the arterial oxygen tension. This effect may be partially mediated *via* reduction of endothelin-1. Aerosolised adrenomedullin could be a potential alternative therapy for pulmonary hypertension, since pulmonary vasodilators having an anti-inflammatory potential and the capability to interact with the upregulated endothelin-1 system could be an innate way to normalise pulmonary vascular tension.

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