

# Catecholamines Reduce Dose-dependent Oedema Formation and Inflammatory Reaction in an Isolated Rat Lung Model

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**Abstract.** *Aim: Since we detected that donor dopamine pre-treatment ameliorates lung function after hypothermia and ischaemia/reperfusion in an isolated rat lung model we studied, whether other catecholamines have beneficial effects on lungs. Materials and Methods: Rats were treated with noradrenaline, adrenaline or dobutamine in different doses. Thereafter lungs were explanted, flushed with Perfadex<sup>®</sup> solution and stored at 4°C for different time periods. Oedema production was measured and inflammatory mediators were analysed after reperfusion and ventilation. Results: Low-dose noradrenaline or dobutamine did not reduce tissue oedema after eight hours of hypothermia, whereas higher doses significantly reduced oedema formation. Low-dose catecholamines did not prevent the inflammatory response, whereas higher doses of beta-receptor-stimulating catecholamines significantly blunted inflammatory reaction. Conclusion: This study demonstrates that adrenergic-receptor-stimulating catecholamines have a protective dose-dependent effect on lungs after hypothermia and ischaemia/reperfusion. Although noradrenaline and dobutamine have similar dose-dependent organ-protective effects to dopamine, they have more side-effects.*

The period of hypothermic and ischemic storage during lung transplantation usually ranges between four and eight hours depending on donor location but is kept as short as possible. Hypothermic organ storage is associated with a decreased

metabolic rate, a reduction of biochemical reactions and 1.5- to 2.0-fold reduced enzyme activity (1). However, a series of events still occur during hypothermia, such as oxidative stress, sodium pump inactivation, iron release and induction of cell death that induce an up-regulation of pro-inflammatory mediators and an activation of recipient leukocytes after reperfusion (2). As leukocytes do, an intact endothelial cell layer and metabolic sequences are necessary to guarantee appropriate lung function after transplantation.

To improve the number of available organs for transplantation, many experimental and clinical studies examine the impact of donor pre-conditioning. Several promising *in vivo* and *in vitro* studies have addressed the improvement of transplant outcome in the donor organ by supplementing preservation solutions with vasodilators *e.g.* sodium nitroprusside (3), nitroglycerine (4) or prostaglandins (5); radical scavengers (6, 7), Platelet-activating factor (PAF)-antagonists (8), anti-inflammatory substances (9), Tumor necrosis factor (TNF)-antagonists (10) or calcium-antagonists (11). Furthermore, whether oxygenation, preservation temperature, reperfusion pressure and ventilation of the transplanted organ could optimize the transplant outcome was analyzed (12-14). Early organ dysfunction after lung transplantation is correlated with warm and cold ischemic time, modes of organ preservation procedures and the extent of donor organ injury. These occur within 72 hours after transplantation and are clinically characterized by non-specific alveolar damage, hypoxaemia, increase of pulmonary artery resistance, and dysfunction of cell membranes including pulmonary oedema formation and leukocyte infiltration (15).

It is known that dopamine significantly reduces cold preservation injury and is able to improve kidney transplantation outcome (16). Benck *et al.* demonstrated that pre-treatment of organ donors with low-dose dopamine improved the clinical outcome of cardiac allograft recipients after transplantation (17). We have proven these described

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Table I. Overview of experimental groups.

	NaCl	DA (5 µg/kg/min)	DB2 (2 µg/kg/min)	DB5 (5 µg/kg/min)	NA0.5 (0.5 µg/kg/min)	NA2 (2 µg/kg/min)	NA5 (5 µg/kg/min)	ADR2 (2 µg/kg/min)	ADR5 (5 µg/kg/min)
n=	9	9	7	7	7	7	8	7	3

dopamine effects in an isolated rat lung model and found significantly less oedema formation after cold preservation with subsequent reperfusion and a significant lower expression of inflammatory mediators (18, 19). The aim of the present study was to analyse if other catecholamines beside dopamine, frequently used in the Intensive Care Unit (ICU), are able to protect lungs from oedema formation. Moreover, the influence of catecholamine pre-treatment on the production of inflammatory mediators, such as cytokine-induced neutrophil chemoattractant-1 (CINC-1), a rat homolog of interleukin-8 (IL-8) and the expression of adhesion molecules was investigated.

## Materials and Methods

**Animals.** Wistar rats weighing 300-350 g were obtained from Janvier®, Rennes, France. Animals were kept under standard conditions and were fed standard rodent chow and water. All procedures were performed according to the Guide for the Care and Use of Laboratory Animals published by the National Academy of Sciences (20) and were approved by German federal regulations (RP Karlsruhe, file no.: 35-9185.81/G-122/07).

**Anaesthesia and experimental protocol.** Animals were anaesthetised by intraperitoneal injection of thiopental (60 mg/kg). An arterial catheter was inserted in the femoral artery to measure the arterial pressure during pre-conditioning time online. A venous catheter was placed in the internal jugular vein to pre-treat the rat for one hour with different catecholamines, dopamine, dobutamine, adrenaline and noradrenaline. During this period the rats were breathing spontaneously.

**Catecholamine-pre-conditioning:** Rats (n=7) received dobutamine at 2 µg/kg/min (DB2) and dobutamine at 5 µg/kg/min (DB5), noradrenaline at 0.5 µg/kg/min (NA0.5), noradrenaline at 2 µg/kg/min (NA2) and noradrenaline at 5 µg/kg/min (NA5) and adrenaline at 2 µg/kg/min (ADR 2) and adrenaline at 5 µg/kg/min (ADR5), intravenously for one hour. NaCl and dopamine at 5 µg/kg/min (DA) pre-treated rats served as control groups (n=9). Mean arterial pressure (MAP) was recorded every ten minutes during pre-conditioning (Table I).

During pre-treatment with NA5 (n=8) and ADR5 (µg/kg/min) (n=3), some of the rats died because of hypertonic crises, with subsequent internal bleeding. We consequently cancelled the experiments in these groups. Five animals survived the pre-conditioning time in NA5 group. Lungs were explanted and reperused directly without cold preservation to analyze CINC-1 in perfusate solution and intercellular-cell-adhesion-molecule-1 (ICAM-1) and vascular-cell-adhesion-molecule-1 (VCAM-1) expression by immunohistochemical staining.

**Operative technique:** In all groups, a 14-gauge vascular catheter was inserted into the trachea by cervical tracheotomy. The animals were ventilated with a small animal respirator, using 95% O<sub>2</sub>/5% CO<sub>2</sub> gas, a tidal volume of 2 ml and a rate of 60 breaths/min with 2-cm H<sub>2</sub>O of positive end-expiratory pressure. Median sternotomy and thymectomy were performed to expose the heart-lung block. After heparin injection into the right ventricle (1000 U/kg), a cannula was placed into the main pulmonary artery through the right ventricular outflow tract and secured with 3-0 silk sutures. The left atrium and ventricle were severed to vent blood. A warm (37°C) modified Krebs-Henseleit buffer (NaCl: 118 mM, KCl: 4.7 mM, KH<sub>2</sub>PO<sub>4</sub>: 1.2 mM, NaHCO<sub>3</sub>: 24 mM, MgSO<sub>4</sub>: 1.2 mM, Glucose: 11.0 mM, CaCl<sub>2</sub>: 1.7 mM and 2% bovine albumine), containing sodium bicarbonate to maintain the pH at 7.3 to 7.4 at 37°C, was used to perfuse the lung. The heart-lung block was mounted in a perfusion chamber and maintained at 37°C. Reperfusion was performed at a constant flow rate of 5 ml/h.

**Cold preservation time and reperfusion:** Cold preservation was carried out by flushing the lungs with 20 ml of cold (4°C) Perfadex® solution (Vitrolife AB, Gothenburg, Germany) through the main pulmonary artery. Thereafter, the heart-lung block was stored at 4°C for six and eight hours and was subsequently reperused at 37°C with warm Krebs-Henseleit solution for different time periods. Lungs not subjected to hypothermic preservation were directly ventilated and perfused for similar time periods. All groups consisted of at least seven animals. Mean pulmonary arterial pressure (PAP) and pulmonary inspiratory pressure (PIP) were measured continuously and recorded every ten minutes (MCG, Hottinger-Baldwin-Messtechnik, Germany). Lung weight was recorded online to assess oedema formation. Remaining lung tissue was frozen in liquid nitrogen and stored at -80°C, or fixed in 4% formaldehyde.

**Immunohistology.** At the end of all experiments, lungs were snap-frozen in liquid nitrogen. Cryostat sections (3-5 µm) were stained by an indirect immunoperoxidase technique. Briefly, ethanol-fixed sections were first incubated with phosphate buffered saline/bovine albumin (PBS)/(BSA) (5% w/v), and subsequently with 2% H<sub>2</sub>O<sub>2</sub>. Thereafter, the sections were incubated for one hour with a primary antibody for ICAM or VCAM (R&D Systems GmbH, Wiesbaden, Germany), followed by extensive washing and were finally incubated with a biotin-conjugated secondary IgG antibody for 1 h. The sections were washed six times in PBS/BSA and incubated with streptavidin-horseradish peroxidase (HRP) for 30 min. Antibody binding was visualised by diaminobenzidine using Vectastain. All sections were counterstained with haematoxylin-eosin. ICAM-1 and VCAM-1 were assessed semiquantitatively using a graded scale from “+” to “++++” (+: very weakly positive, ++: weakly positive, +++: positive, ++++: strongly positive). More than 20 fields of vision were blindly evaluated under a microscope at a magnification of 40.

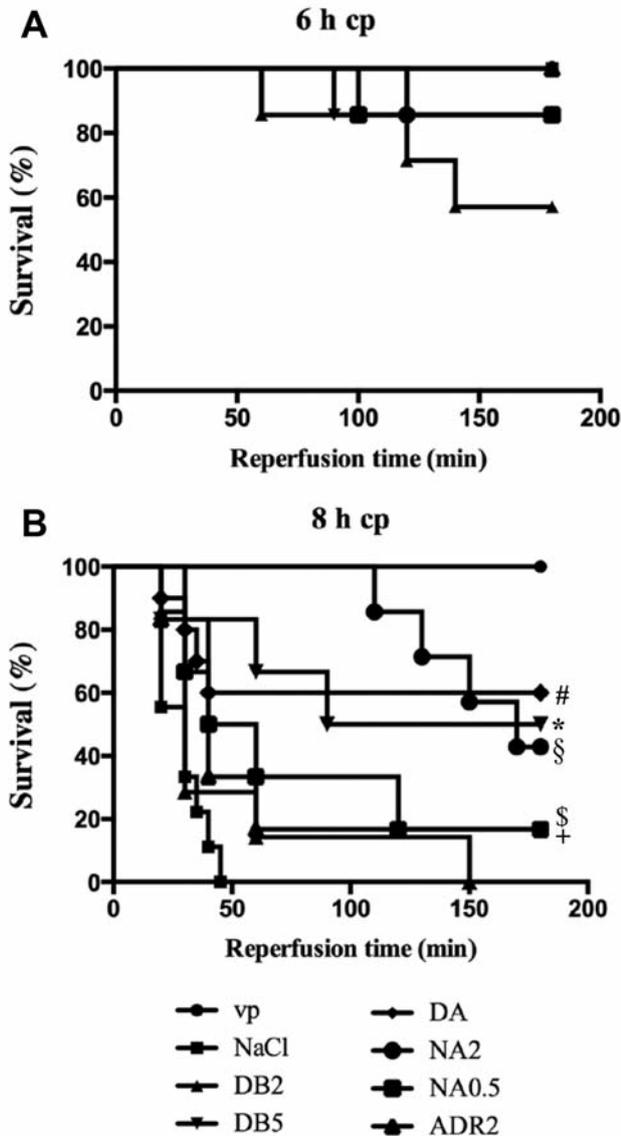


Figure 1. Influence of adrenaline, noradrenaline and dobutamine treatment on formation of pulmonary oedema. Rats were pre-treated with adrenaline (ADR), noradrenaline (NA) and dobutamine (DB) 1 h before lung explantation. NaCl and dopamine (DA)-treated rats served as control. The lungs were subjected to cold preservation (cp) for 6 h (A) or 8 h (B), subsequently reperfused with warm Krebs-Henseleit solution and ventilated for 180 min. Lungs not subjected to cold preservation served as additional control. Kaplan Meier analysis was performed using weight increase more than 4 g as an end-point. After 6 h of cold preservation no significant differences were seen. Significant differences between the groups were only observed after 8 h of cold preservation.: NaCl vs. DA  $^{\#}p < 0.005$  (15), NaCl vs. DB5  $^{*}p < 0.002$ , NaCl vs. NA2  $^{\$}p < 0.0001$ , DA vs. DB2  $^{\$}p < 0.05$ , DA vs. NA0.5  $^{+}p < 0.05$  (by log-rank-test).

**CINC-1.** CINC-1 concentration was measured in the perfusion solutions by enzyme-linked immunosorbent assay (ELISA) (R&D Systems GmbH, Wiesbaden, Germany). Sensitivity of the ELISA was  $< 0.08$  pg/ml for CINC-1. Each experiment was performed at

least five times and the CINC-1 concentration for each individual sample was assessed in triplicate.

**Statistical analysis.** Data are shown as the mean  $\pm$  SD. Comparisons were made using Wilcoxon's rank sum test and the Mann-Whitney U-test. GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA) was used for statistical analysis.  $p$ -Values of  $p < 0.05$  were considered significant.

## Results

**Influence of catecholamine pre-treatment on oedema formation after cold preservation and during reperfusion.** To investigate the influence of cold preservation on oedema formation, lungs from ADR2-, NA0.5-, NA2-, NA5-, DB2- and DB5-treated rats were explanted and stored in Perfadex<sup>®</sup> solution for six and eight hours, subsequently perfused for 180 min with warm Krebs-Henseleit solution and ventilated for three hours. Lungs pre-treated with DA or NaCl were used as positive controls, lungs without cold preservation served as negative controls [ventilated and perfused (vp)]. When cold preservation time was six hours, the increase in lung weight did not significantly differ between lungs that were subjected to cold preservation and lungs that were not (Figure 1A).

After eight hours of cold preservation time (Figure 1B), lung weight increased within 35 min of reperfusion time in the NaCl control group. Oedema formation was clearly shown after 150 min in all DB2-pretreated lungs. This was in contrast to DA treatment, where reperfusion time without oedema formation lasted significantly longer. NA2 and DB5 pre-treatment significantly reduced oedema formation in a DA-like manner. ADR2 and NA0.5 treatment resulted in less oedema than NaCl treatment and showed a tendency towards protection in these lungs.

**Influence of cold preservation on inflammatory mediators after pre-treatment with catecholamines.** To study if catecholamine pre-conditioning might affect the inflammatory response after reperfusion, we assessed the production of CINC-1 in the perfusate of lungs after 30 and 180 min of reperfusion. NaCl and DA-pre-treated lungs served as controls.

After 30 min, no CINC-1 production was seen at all. In lungs pre-treated with NaCl, DB2, NA0.5 and ADR2, CINC-1 was clearly detectable in the perfusate three hours after reperfusion. As hypothetically argued, this was significantly inhibited by DA, but also NA2, NA5 and DB5 pre-treatment significantly blunted CINC-1 production (Figure 2).

To investigate the expression of adhesion molecules, immunohistochemical staining for ICAM-1 and VCAM-1 was conducted (Table II). As shown in Figure 3, an up-regulation of ICAM-1 was clearly pronounced in NaCl pre-treated lungs (control) without cold preservation. ADR2- and

Table II. Immunohistological staining of lungs for intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) after pretreatment with adrenaline (ADR), noradrenaline (NA) or dobutamine (DB) for 1 hour without or after cold preservation. NaCl and dopamine (DA) served as control. Rat lungs were directly reperfused without cold preservation (no cp), 6 hours cold preservation (6 h cp) or 8 hours cold preservation (8 h cp) with subsequent reperfusion for 3 hours. Results are expressed semi-quantitatively (+: very weakly-positive, ++: weakly-positive, +++: positive, ++++: strongly-positive).

Condition	No cp		6 h cp		8 h cp	
	ICAM-1	VCAM-1	ICAM-1	VCAM-1	ICAM-1	VCAM-1
NaCl	++++	++++	++	++	++	++
DA	+	++	++	+++	++	+++
DB2	++++	++++	++	+++	++	++
DB5	++	++	+++	+++	+++	++
NA0.5	++	+++	++++	++++	++	++
NA2	+	++	++	+++	++	++
ADR2	++++	+++	+++	+++	++	+++

DB2-pre-treated lungs also exhibited a high expression of ICAM-1. Adhesion molecule expression was significantly lower after DA-pre-treatment. Moreover NA0.5, NA2, NA5 and DB5 significantly reduced adhesion molecule expression.

The production of CINC-1, but also the up regulation of adhesion molecules ICAM-1 and VCAM-1 were significantly reduced by pre-treatment with higher doses of DB and NA. Lower doses of DB and NA, as well as ADR, had no significant effect on inflammation in these lungs.

### Discussion

Primarily, the results of the present study confirm that donor stimulation of beta-adrenergic receptors by catecholamines leads to a significant reduction of oedema formation upon reperfusion in lungs after eight hours of cold ischaemia. Moreover, pre-treatment with beta-adrenergic receptor-stimulating catecholamines significantly reduces the production of inflammatory mediators, such as cytokines and adhesion molecules, in rat lungs after reperfusion. These effects were seen in a substance- and dose-dependent manner. But neither noradrenaline nor dobutamine led to better effects than low-dose dopamine.

*Influence of catecholamines on pulmonary oedema.* The balance between formation and re-absorption of alveolar fluid volume is called alveolar fluid clearance. If alveolar fluid formation overwhelms the clearance capacity, the resulting alveolar oedema causes deterioration of gas exchange across the alveolar epithelium and leads to acute respiratory failure. Ion gradients generated by transported ions drive fluid out of the alveolar spaces (21). At the molecular level, adrenergic (alpha or beta) receptor-mediated effects are based on cAMP-dependent activation or suppression of protein kinase A (PKA) or C (PKC), and on affecting the activation of cAMP

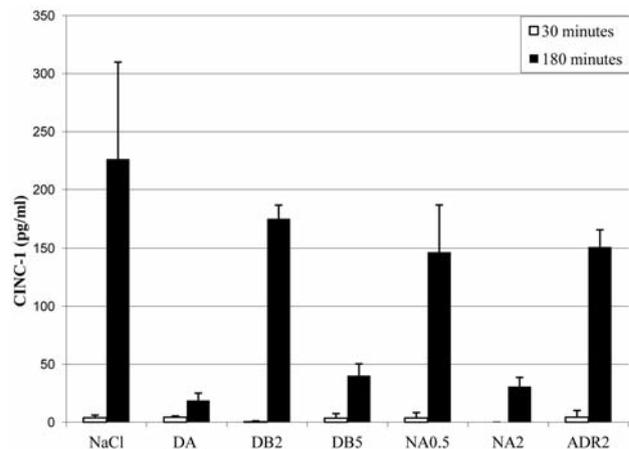
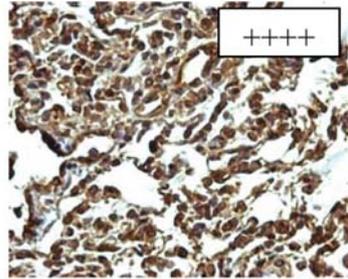


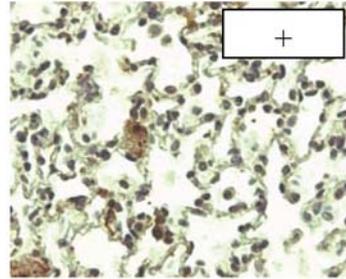
Figure 2. Influence of cold preservation on Cytokine-induced neutrophil chemoattractant-1 (CINC-1) production after pre-treatment with adrenaline, noradrenaline and dobutamine. Rats were treated with adrenaline (ADR), noradrenaline (NA) and dobutamine (DB) as described in Materials and Methods, or left untreated (NaCl), or were pre-treated with dopamine (DA) as control groups. Thereafter the lungs were directly ventilated and perfused with warm Krebs-Henseleit solution for 180 min. CINC-1 concentration was measured in the perfusate solution after 30 min of reperfusion time and at the end of the reperfusion period. Each group consisted of five animals. The results are expressed as mean CINC-1 (pg/ml)  $\pm$ SD. Significant differences were seen between NaCl vs. DB5  $p < 0.0001$ , NaCl vs. NA2  $p < 0.01$ , NaCl vs. NA5  $p < 0.0005$ , DA vs. DB2  $p < 0.005$ , DA vs. NA0.5  $p < 0.01$ , DA vs. ADR2  $p < 0.01$ .

response element-binding protein (CREB) and/or nuclear factor-kappa B (NF- $\kappa$ B) (22). Our control lungs presented immediate oedema formation at the beginning of reperfusion after eight hours of hypothermia and reperfusion. Catecholamine pre-treatment minimized this oedema

**References:**

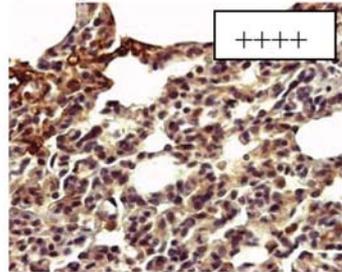


NaCl

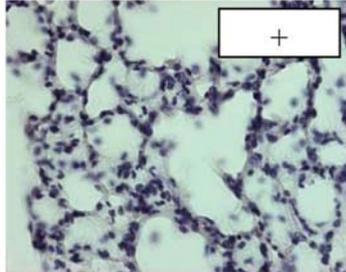


DA

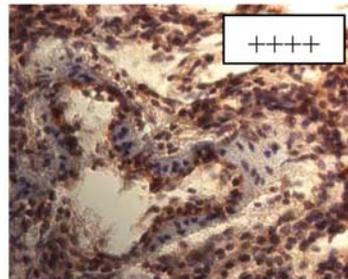
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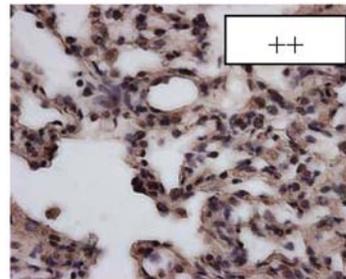
ADR2



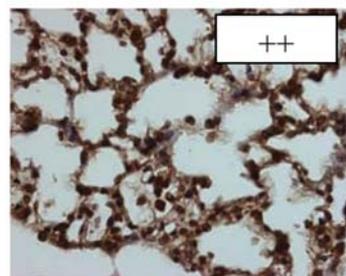
NA2



DB2



NA0.5



DB5

Figure 3. Influence of adrenaline, noradrenaline and dobutamine treatment on ICAM expression. Rats were treated as described in Materials and Methods. Thereafter the lungs were directly reperfused and ventilated for 180 min. The result of a representative experiment (n=5) is depicted (+: very weakly-positive, ++: weakly-positive, +++: positive, ++++: strongly positive). Magnification  $\times 40$ .

formation in a dose-dependent manner when concentrations preferentially stimulating beta-adrenergic receptors were used (NA and DB in higher doses). As we demonstrated in a previous study, this effect was adrenoceptor-mediated (19).

There is controversy as to which receptor mediates alveolar clearance. It has been reported that NA reduces lung fluid production by the activation of alpha-adrenergic receptors (23, 24) and that beta-adrenergic receptor stimulation

increases lung oedema clearance by up-regulation of Na<sup>+</sup>-channels and Na<sup>+</sup>/K<sup>+</sup>-ATPase (25-28). We demonstrated in the present study that even after eight hours of cold preservation the stimulation of beta-receptors by NA and DB leads to significantly reduced oedema formation upon reperfusion in a dose-dependent manner. Lower doses of NA, DB and ADR (more stimulation of alpha-receptors) did not induce a significant effect. In several rat lung studies, Sznajder *et al.* demonstrated that increased sodium channel and Na<sup>+</sup>/K<sup>+</sup>-ATPase activity stimulate lung fluid clearance and this is mediated by stimulation of beta receptors (29, 30). In contrast Xu *et al.* postulated that alveolar fluid clearance is mediated *via* alpha-adrenoceptors in a rat model of acute pancreatitis (31), whereas Azzam reported an increased lung fluid reabsorption *via* activation of both, alpha1- and beta-receptors, but not alpha2-receptors (32). This was in accordance with our previous study where we found that alpha- and beta-blockade of adrenergic receptors resulted in immediate oedema formation after eight hours of cold preservation and reperfusion (19).

*Influence of catecholamines on inflammation.* Several clinical and experimental studies documented a rapid release of proinflammatory cytokines in kidneys (33), liver (34), heart (35) and lung (18, 36) after ischaemia and reperfusion. Whereas most cytokine levels decreased after reperfusion, the chemokine IL-8 significantly increased after reperfusion and correlated negatively with graft function (37). Fisher (38) and de Perrot (37) demonstrated that high levels of IL-8 in donor lung tissue or bronchoalveolar lavage are associated with an increased risk of death from primary graft dysfunction after transplantation. Another group reported that after two hours of ischaemia, lung injury could be prevented by monoclonal antibody treatment against IL-8 (38, 40). Whereas binding to the alpha-receptor is associated with predominantly immunostimulatory effects (*e.g.* induction of TNF alpha and IL-1 beta), stimulation of the beta-receptors has usually immunosuppressive consequences (*e.g.* inhibition of TNF alpha and IL-1 beta, induction of IL-10). Stimulation of both, alpha- and beta-receptors results in predominance of beta-receptor stimulation (41). This is in concordance with our findings that stimulation of alpha-adrenergic receptors by low doses of NA and DB caused an increase in CINC-1 production during reperfusion. However, stimulation of beta adrenergic receptors by higher doses of NA and DB significantly reduced the expression of CINC-1.

The endothelial barrier function is important to prevent vascular leakage and free migration of inflammatory cells into the interstitium with subsequent oedema formation (42). Adhesion molecules are up-regulated on endothelial cells during ischaemia and reperfusion, such as ICAM-1 and VCAM-1 expression by IL-1 and TNF- $\alpha$  (43, 44). Blockade of adhesion molecules at the time of reperfusion results in

reduced lung reperfusion injury (45, 46). Our findings support this as lungs with reduced adhesion molecule expression also exhibited significantly less oedema formation and CINC-1 production. Higher levels of adhesion molecules are accompanied by significant lung oedema formation and production of CINC-1. These effects seem to be adrenergic receptor-mediated as adrenergically stimulated lungs exhibit significantly lower levels of ICAM-1 and VCAM-1. However, adrenoceptor blockade leads to an up regulation of adhesion molecules, as we have demonstrated previously (19).

In conclusion, our study demonstrates that pre-treatment with catecholamine-stimulating beta-adrenoceptors, significantly limits the formation of pulmonary oedema in a dose-dependent manner during reperfusion of lungs subjected to prolonged cold preservation. Moreover, beta-receptor-stimulating substances act as anti-inflammatory agents under these conditions, as they inhibit CINC-1 production and the expression of adhesion molecules. But neither NA nor DB seem to be more effective than DA. Additionally, the tested catecholamines have a higher risk of side effects, such as haemodynamical derangement with corresponding consequences, as we saw in rats that died from internal bleeding after ADR5 or NA5 pretreatment. To utilise the protective immunomodulatory effect of catecholamines in clinical routine, more studies need to be conducted to develop new substances without haemodynamic side-effects to protect the available donor organs much better.

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