Adenosine A(1) and A(2) receptor agonists reduce endotoxin-induced cellular energy depletion and oedema formation in the lung

Axel R. Heller, J. Rothermel, M.A. Weigand, K. Plaschke, J. Schmeck, Martina Wendel, H.J. Bardenheuer, Thea Koch

Angaben zur Veröffentlichung / Publication details:

Heller, Axel R., J. Rothermel, M.A. Weigand, K. Plaschke, J. Schmeck, Martina Wendel, H.J. Bardenheuer, and Thea Koch. 2007. "Adenosine A(1) and A(2) receptor agonists reduce endotoxin-induced cellular energy depletion and oedema formation in the lung." *European Journal of Anaesthesiology* 24 (3): 258–66. https://doi.org/10.1017/S026502150600144X.

Adenosine A_1 and A_2 receptor agonists reduce endotoxin-induced cellular energy depletion and oedema formation in the lung

A. R. Heller[*], J. Rothermel[†], M. A. Weigand[¶], K. Plaschke[¶], J. Schmeck[†], M. Wendel[*], H. J. Bardenheuer[¶], T. Koch[*]

[*]*University Hospital Carl Gustav Carus, Harvard Medical International Associated Institution, Department of Anesthesiology and Intensive Care Medicine, Dresden;* [†]*University Hospital, Department of Anesthesiology and Intensive Care Medicine, Mannheim;* [¶]*Ruprecht-Karls University Heidelberg, Department of Anesthesiology and Intensive Care Medicine, Germany*

Summary

Background and objective: Tissue depletion of adenosine during endotoxaemia has previously been described in the lung. Therapeutic approaches to prevent adenosine depletion and the role of A_1 and A_2 receptor agonists, however, have not been investigated until now. *Methods:* In isolated and ventilated rabbit lungs, it was tested whether pretreatment with adenosine A_1 agonist 2-chloro-*N*6-cyclopentyladenosine (CCPA; 10^{-7} mol, $n = 6$) or A_2 receptor agonist 5′-(*N*-cyclopropyl)-carboxyamido adenosine (CPCA; 10^{-7} mol, $n = 6$) prior to injection of lipopolysaccharide (LPS) (500 pg mL^{-1}) influenced pulmonary artery pressure (PAP), pulmonary energy content and oedema formation as compared with controls, solely infused with LPS ($n = 6$). Release rates of adenosine and uric acid were determined by high-performance liquid chromatography. Pulmonary tissue concentrations of high-energy phosphates were measured and the adenine nucleotide pool, adenosine 5′-triphosphate (ATP)/adenosine 5′-diphosphate (ADP) ratio and adenylate energy charge of the pulmonary tissue were calculated. *Results:* Administration of LPS induced increases in PAP within 2 h up to 20.8 ± 2.9 mmHg ($P < 0.01$). While pretreatment with the A_1 agonist merely decelerated pressure increase (13.8 ± 1.1 mmHg, $P < 0.05$), the A_2 agonist completely suppressed the pulmonary pressure reaction (9.6 ± 1.0 mmHg, $P < 0.01$). Emergence of lung oedema after exclusive injection of LPS up to 12.0 ± 2.9 g was absent after A_1 (0.6 ± 0.5 g) and A_2 (-0.3 ± 0.2 g) agonists. These observations were paralleled by increased adenosine release rates compared with LPS controls ($P < 0.05$). Moreover, tissue concentrations of ADP, ATP, guanosine 5′-diphosphate, guanosine 5′-triphosphate, nicotinamide-adenine-dinucleotide and creatine phosphate were significantly reduced after LPS. Consequently, the calculated tissue adenine nucleotide pool and the adenylate energy charge increased after adenosine receptor stimulation ($P = 0.001$). *Conclusions:* Adenosine A_1- and A_2-receptor agonists reduced LPS-induced vasoconstriction and oedema formation by maintenance of tissue energy content. Thus, adenosine receptor stimulation, in particular of the A_2 receptor, might be beneficial during acute lung injury.

Keywords: NEUTROPHILS; ENDOTHELIUM; INFLAMMATION; ADENOSINE, receptors; ADENOSINE TRIPHOSPHATE; ACUTE RESPIRATORY DISTRESS SYNDROME; RABBIT; ENDOTOXIN.

Correspondence to: Axel R. Heller, Klinik und Poliklinik für Anaesthesiologie, Universitätsklinikum Carl Gustav Carus, Fetscherstrasse 74, D-01309 Dresden, Germany. E-mail: axel.heller@mailbox.tu-dresden.de; Tel: +49 351 458 2785; Fax: +49 351 458 4336

Introduction

During endotoxaemia, the lung is depleted of energy-rich adenine nucleotides (up to 38% of ATP) [1]. Correspondingly, haemodynamic [2] and pulmonary conditions [3] improved in endotoxaemic pigs when adenosine was administered. Moreover, pretreatment with adenosine reversed neutrophil-induced pulmonary oedema in isolated dog lungs [4]. The latter effects have been explained by investigators by reduced levels of pro-inflammatory lipid mediators such as thromboxane A_2 or leukotrienes [5]. Protective effects of adenosine on vascular endothelium were associated with lower levels of vascular endothelial growth factor [6].

Adenosine A_1 receptor activation induces the release of vasoconstrictive thromboxane A_2, and the inhibition of adenylate cyclase (AC), and promotes neutrophil (PMN) chemotaxis and adherence to the endothelium [5]. In contrast, anti-inflammatory effects of adenosine were attributed to adenosine A_{2a}, A_{2b} and A_3 receptor mediated reduction of PMN chemotaxis [7], PMN adhesion [8], transendothelial migration [6] and inflammatory mediator generation [9]. Sub-threshold inflammatory stimuli were, vice versa, sufficient to induce extensive tissue damage in adenosine A_{2a} receptor knock-out mice [10] and adenosine receptor antagonists depressed hepatosplanchnic blood flow [11].

Decreased tissue content of high-energy phosphates and the release of their metabolites indicate ischaemia and cellular hypoxia [12,13]. Under these conditions, hypoxanthine and uric acid (UA) are generated by xanthine oxidase, which has been demonstrated to mediate endotoxin (lipopolysaccharide (LPS))-induced hyperpermeability [14].

The current study was performed to investigate whether selective adenosine receptor agonists may counterbalance lung tissue depletion of energy-rich adenine nucleotides during LPS-induced acute lung injury. Therefore, lungs were challenged with LPS in the presence or absence of adenosine A_1 agonist 2-chloro-*N*6-cyclopentyladenosine (CCPA) or adenosine A_2 receptor agonist 5′-(*N*-cyclopropyl)-carboxyamido adenosine (CPCA). We measured pulmonary artery pressure (PAP), oedema formation, the pulmonary release of adenosine and UA into the perfusate, the pulmonary tissue concentrations of the cellular energy carriers or storage forms adenosine 5′-triphosphate (ATP), adenosine 5′-diphosphate (ADP), adenosine 5′-monophosphate (AMP), creatine phosphate (CrP), adenosine, guanosine 5′-triphosphate (GTP), guanosine 5′-diphosphate (GDP), guanosine 5′-monophosphate (GMP) and nicotinamide-adenine-dinucleotide (NAD) [15].

Materials and methods

Isolated rabbit lung

The techniques of preparing and perfusing isolated rabbit lungs have been previously described in detail [16–18]. After approval by the local board for animal protection, female chinchilla rabbits (*Orticolagus caniculus*) weighing 2100 ± 196 g (mean ± standard deviation) were anaesthetized with ketamine ($50\ mg\ kg^{-1}$) and xylazine ($4\ mg\ kg^{-1}$) and anticoagulated with heparin-sodium $1000\ U\ kg^{-1}$, injected in the ear vein. After placement of a tracheostomy tube, the rabbits were mechanically ventilated with room air. The thorax was opened via the diaphragm and after a median sternotomy a catheter was inserted into the pulmonary artery. The lung organ preparation was isolated and suspended from a force transducer in a temperature-controlled (37°C) and humidified chamber. After the cannulation procedure, the lungs were perfused with 200 mL Krebs Henseleit hydroxyethyl starch buffer solution (KHB) by a peristaltic pump at a constant volume inflow of $150\ mL\ min^{-1}$ in a closed recirculatory system with an open reservoir to collect outflow from the left atrium. Pulmonary embolism by circulating particles or air was prevented by a 40 μm filter and a bubble trap placed just before the pulmonary artery catheter. The lungs were ventilated with 4% CO_2 in air (frequency $25\ min^{-1}$, tidal volume 25 mL, positive end-expiratory pressure (PEEP) 0.5–1.0 cm H_2O) and, in order to avoid atelectasis formation, intermittently expanded by increasing the expiratory pressure up to 3 cm H_2O for three inspirations. The mean PAP and pulmonary airway pressures (PAW) were continuously recorded via Statham strain gauge transducers. Due to a constant perfusion flow, alterations of perfusion pressure directly reflect alterations of pulmonary vascular resistance. Intermittently, samples of perfusate were taken from a catheter, which collected the effluent from the pulmonary veins, for measurements of pH (blood gas analysis system 288, Ciba Corning, Fernwald, Germany) and oncotic pressure (Onkometer BMT 921, Dr Karl Thomae GmbH, Germany) as well as for the determination of adenosine and UA release. Initially the lungs were rinsed with KHB solution, using low flow rates in an open circulatory system to remove remaining 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 15 min later, after the flow was stepwise increased to $150\ mL\ min^{-1}$. After another 30 min steady-state period, these lungs had a constant mean PAP of 8–9 mmHg (zero-referenced at the hilum). The only

lungs selected for the study were those that showed a homogeneous white appearance with no signs of haemostasis or oedema formation, and which had no changes in weight during the steady-state period. In previous experiments, the perfusion with KHB has been documented to maintain integrity of the micro-circulation for more than 5 h in our model, which was assessed by measurements of PAP and weight gain, by biochemical analysis (lactatedehydrogenase, AA-metabolites and histamine) and by ultrastructural studies [17].

Experimental protocol

Eighteen lungs were randomly assigned to three experimental groups. Ten minutes after the final perfusion flow (150 mL min^{-1}) was reached and stable haemodynamics were observed, all lungs received autologous rabbit blood (final concentration 10% in perfusate), which was drawn during the preparation phase. Ten minutes later either the adenosine A_1 receptor agonist CCPA or the adenosine A_2 receptor agonist CPCA was added to the perfusate. Six additional lungs served as controls. Following a further 10 min period, LPS was administered, yielding final concentrations of 500 pg mL^{-1}. Perfusate samples were drawn 5 min prior to administration of CCPA or CPCA (15 min), before (0) and 15, 30, 60 and 120 min after LPS. Collecting sites were the pulmonary artery and simultaneously the left atrium. This procedure enabled calculation of release rates of adenosine and UA from the lung.

Perfusion buffer

The perfusate consisted of a KHB 10% blood buffer solution with a colloid oncotic pressure of 23–25 mmHg, yielding final concentrations of Na^+ 138 mmol L^{-1}; K^+ 4.5 mmol L^{-1}; Mg^{2+} 1.33 mmol L^{-1}; Cl^- 135 mmol L^{-1}; Ca^{2+} 2.38 mmol L^{-1}; glucose 12 mmol L^{-1}; HCO_3^- 12 mmol L^{-1}. The osmolality was approximately 330 mosm kg^{-1} (Mikro-Osmometer, Roebling Meßtechnik, Berlin, Germany). The pH of the buffer solution was adjusted to 7.4 with 1 mol $NaHCO_3$.

Endotoxin

A trichloroacid preparation of LPS from *Escherichia coli 0111*, extracted according to the Boivin method, was provided by Dr R. Urbaschek (Department of Microbiology, University Hospital, Mannheim, Germany). LPS aliquots were stored deep-frozen at −20°C until use. The concentration of LPS (500 pg mL^{-1}) was chosen according to previously published work by our group [18]. This concentration is in line with clinically reported concentrations in Gram-negative septic shock [19] and represents a reproducible inflammatory stimulus in this model.

Adenosine receptor agonists

The adenosine receptor agonists CCPA (Lot: LR-III-33) and CPCA (Lot: TB-491B) were purchased from Sigma-Aldrich (Deisenhofen, Germany). A stock solution of 1 mg mL^{-1} in dimethylsulphoxide (DMSO, Merck, Darmstadt, Germany) was further diluted with saline to receive respective final concentrations in the perfusate (CCPA 10^{-7} mol, CPCA 10^{-7} mol), which are in accordance with published doses [20].

Analysis of purine compounds

The purine compounds adenosine and UA were measured in the perfusate (1 mL), according to the method of Bardenheuer and colleagues [21]. Nucleoside uptake by red blood cells in the sample was prevented by pre-cooled dipyridamole solution (0.2 mL; 5×10^{-5} mol). After immediate centrifugation at 4°C, plasma supernatant (0.3 mL) was deproteinated with perchloric acid (70%; 0.05 mL). After neutralization (KH_2PO_4) and centrifugation, nucleosides were determined by high-performance liquid chromatography (HPLC). We automatically injected 0.1 mL samples onto a C-18 column (Nova-Pak C18, 3.9 mm × 150 mm, Waters Instruments, Rochester, NY, USA). The linear gradient started with 100% KH_2PO_4/K_2HPO_4 1 : 1 (0.1 mol, pH 4.0) and increased to 60% of 60 : 40 methanol/water (v/v) in 15 min, the flow rate being 1.0 mL min^{-1}. This was followed by a reversal of the gradient to initial conditions over the next 3 min. We continuously monitored absorbance of the column eluate by using a photodiode array detector (Type 996, Waters Instruments, Rochester, NY) to measure adenosine at 254 nm and UA at 293 nm. We performed peak identification and quantification of the respective compounds by comparing the retention times of the sample peaks with respective peaks of ultra-pure standards by using a computer-assisted program (Waters, Millenium). Since a recirculatory lung perfusion system was used, release rates of purines into the perfusate were calculated from the difference of simultaneous pulmonary arterial and pulmonary venous measurements, considering a perfusate flow rate of 2.5 mL s^{-1}.

Tissue content of high-energy phosphates/metabolites

At the end of the 120 min study period, ATP, ADP, AMP, adenosine, GTP, GDP, GMP, NAD and CrP

were determined in a shock-frozen segment of the lung at hilum level, by HPLC, after disruption of cell membranes with an ultraturrax in a chloroform/acetic acid mixture (1 : 2) at −20°C. After neutralization (potassium hydroxide) and centrifugation, 0.1 mL of the supernatant was then used for HPLC analysis with a Partisil SAX column (4.6 mm inside diameter × 250 mm, 10 μm pore size). The mobile phase was changed from 100% 0.01 mol H_3PO_4 to 100% 0.75 mol KH_2PO_4 by a linear gradient over 45 min. Within the next 3 min, the gradient was reversed to 100% 0.01 mol H_3PO_4, and the next sample was injected after 12 min of re-equilibration at that level. The flow rate was 1.0 mL min^{-1}, and ATP, ADP, AMP, adenosine, GTP, GDP, GMP, NAD and CrP were simultaneously detected by a photodiode array detector (Waters Instruments) at 254 and 210 nm. From these data, the adenine nucleotide pool (ATP + ADP + AMP), the ATP/ADP ratio and the adenylate energy charge [(ATP + 0.5 ADP)/(ATP + ADP + AMP)] in the pulmonary tissue were calculated [12,22].

Statistical analysis and data presentation

Data are presented as means ± standard errors of the mean (SEM). Differences between groups were tested by one-way analysis of variance (ANOVA) followed by a Bonferroni's test for multiple comparisons. Observations between groups (between-subject factor) over time (within-subject factor) were tested with the general linear model (GLM) for repeated measurements, according to a two-way ANOVA. Statistical significance was accepted for all procedures at $P < 0.05$. Statistical analysis was performed with SPSS software for MS Windows (Release 10.0.7, SPSS Inc., Chicago, IL, USA).

This study was approved by the Animal Subject Protection Committee of the local government. The care and handling of animals were in accordance with the principles expressed in the Helsinki Declaration.

Results

Haemodynamic data and lung oedema

Following a 30 min steady-state phase, baseline values of PAP ranged between 8 and 11 mmHg in all groups (airway pressure 6–8 cm H_2O), according to previous data [16–18]. Inflammatory stimulation with LPS increased PAP (Fig. 1) within 120 min up to 20.8 ± 2.9 mmHg ($P < 0.01$). Pretreatment of the lungs with the adenosine A_1 agonist CCPA significantly delayed pressure increase ($P < 0.05$). The adenosine A_2 agonist CPCA completely suppressed

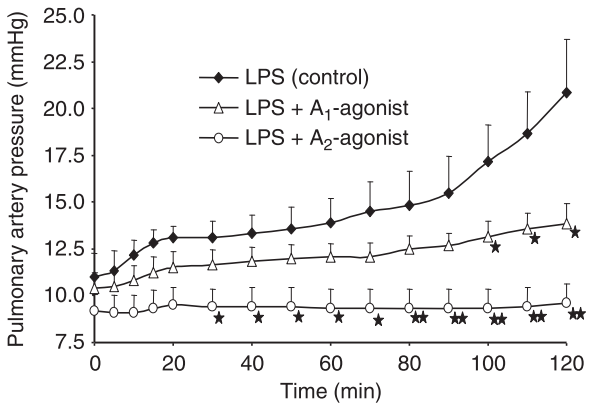

Figure 1.
PAP (mean ± SEM) of isolated rabbit lungs after endotoxin (LPS) 500 pg mL^{-1} in the presence of adenosine receptor A_1 agonist (CCPA, 10^{-7} mol, n = 6); A_2 receptor agonist (CPCA, 10^{-7} mol, n = 6) or in controls (LPS, n = 6). $^{*}P < 0.05$; $^{**}P < 0.01$ vs. LPS, ANOVA.

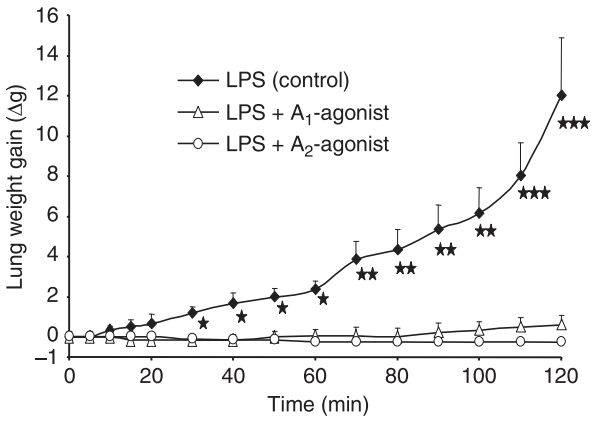

Figure 2.
Pulmonary oedema formation expressed as weight gain [Δg] (mean ± SEM) in isolated rabbit lungs after endotoxin (LPS) 500 pg mL^{-1} in the presence of adenosine receptor A_1 agonist (CCPA, 10^{-7} mol, n = 6); A_2 receptor agonist (CPCA, 10^{-7} mol, n = 6) or in controls (LPS, n = 6). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ vs. LPS, ANOVA.

PAP increase until the end of the observation period ($P < 0.01$).

In positive controls, LPS stimulation pulmonary oedema occurred as indicated by a weight increase of 12.0 ± 2.9 g until the end of the experiment (Fig. 2). Both CCPA and CPCA completely suppressed lung-oedema formation ($P < 0.001$).

Purine compounds

Baseline values of adenosine ranged between 200 and 210 nmol L^{-1}, whereas UA was not detectable. Adenosine release rates were calculated as 300 pmol s^{-1}, which reflects an uptake of adenosine into pulmonary tissue. Administration of both

adenosine A_1 and adenosine A_2 receptor agonists induced an inversion of total adenosine flux (Fig. 3; CCPA 325 ± 109 pmol s^{-1}, CPCA 69 ± 43 pmol s^{-1}). Until the end of the observation period, adenosine release under CCPA and CPCA was found to be significantly ($P < 0.05$) elevated as compared with controls. The effects of CCPA and CPCA on concentrations of UA to 1.6 ± 0.5 μmol and 1.5 ± 0.7 μmol, respectively, compared with LPS (1.0 ± 0.4 μmol) failed to be statistically significant.

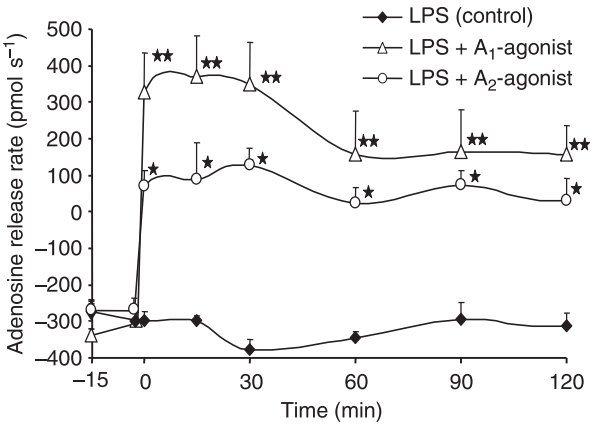

Figure 3.
Adenosine release [pmol s^{-1}] (mean ± SEM) into the perfusate of isolated rabbit lungs after endotoxin (LPS) 500 pg mL^{-1} in the presence of adenosine receptor A_1 agonist (CCPA, 10^{-7} mol, n = 6); A_2 receptor agonist (CPCA, 10^{-7} mol, n = 6) or in controls (LPS, n = 6). $^{*}P < 0.05$; $^{**}P < 0.01$ vs. LPS, ANOVA.

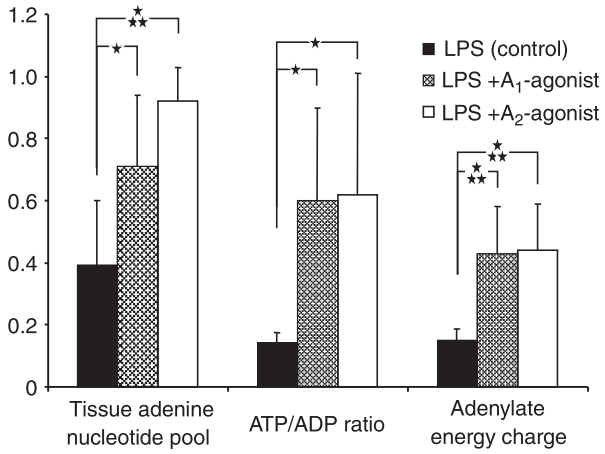

Figure 4.
Tissue-adenine nucleotide pool [μmol g^{-1}], ATP/ADP ratio [mol mol^{-1}] and adenylate energy charge [mol mol^{-1}] (mean ± SEM) in isolated rabbit lungs after endotoxin (LPS) 500 pg mL^{-1} in the presence of adenosine receptor A_1 agonist (CCPA, 10^{-7} mol, n = 6); A_2 receptor agonist (CPCA, 10^{-7} mol, n = 6) or in controls (LPS, n = 6). $^{*}P < 0.05$; $^{***}P < 0.001$ vs. LPS, ANOVA.

Tissue content of high-energy phosphates/metabolites

The effects of endotoxaemia in the presence or absence of adenosine A_1 or A_2 agonists on the concentrations of ATP, ADP, AMP, GTP, GDP, GMP, CrP and NAD in the lung tissue are shown in Figures 4–6. In controls (Group LPS), significantly decreased tissue concentrations of ADP, ATP, GDP, GTP, NAD and CrP were observed, compared with animals that additionally received adenosine A_1 or A_2 agonists ($P < 0.05$). Due to a high degree of variation in adenosine and AMP tissue levels, no significant group differences were observed. The tissue adenine nucleotide pool in the lung (Fig. 4)

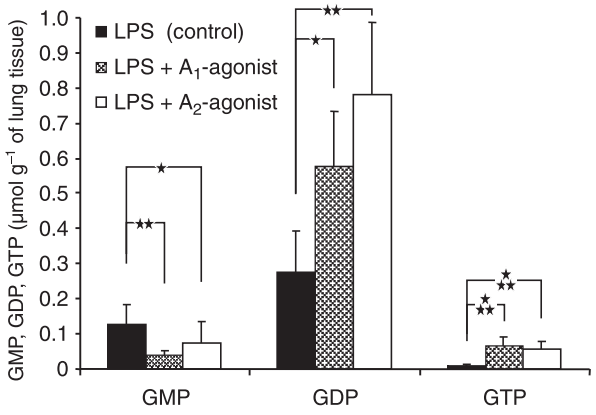

Figure 5.
Tissue concentrations [μmol g^{-1}] (mean ± SEM) of GMP, GDP and GTP) in isolated rabbit lungs after endotoxin (LPS) 500 pg mL^{-1} in the presence of adenosine receptor A_1 agonist (CCPA, 10^{-7} mol, n = 6); A_2 receptor agonist (CPCA, 10^{-7} mol, n = 6) or in controls (LPS, n = 6). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ vs. LPS, ANOVA.

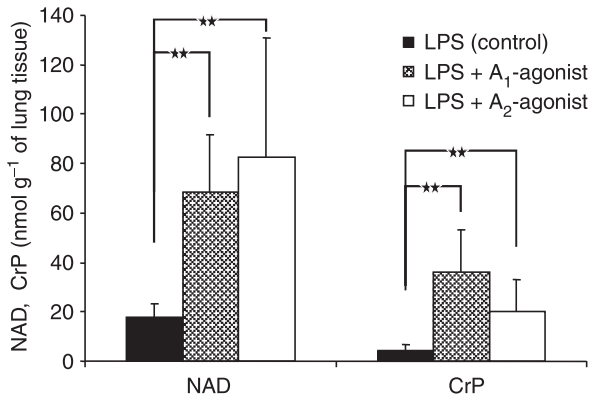

Figure 6.
Tissue concentrations [nmol g^{-1}] (mean ± SEM) of NAD and CrP in isolated rabbit lungs after endotoxin (LPS) 500 pg mL^{-1} in the presence of adenosine receptor A_1 agonist (CCPA, 10^{-7} mol, n = 6); A_2 receptor agonist (CPCA, 10^{-7} mol, n = 6) or in controls (LPS, n = 6). $^{**}P < 0.01$ vs. LPS, ANOVA.

in controls was decreased compared with animals receiving A_1 ($P < 0.05$) or A_2 agonists ($P < 0.001$). Moreover, both the adenylate energy charge and the ATP/ADP ratio were significantly elevated after administration of A_1 or A_2 agonists.

Taken together, during endotoxaemia adenosine A_1 and adenosine A_2 receptor agonists preserved the tissue concentrations of all high-energy nucleotides and, consequently, the adenine- and guanosine-nucleotide pool in the lung.

Discussion

In the course of critical illness, systemic inflammatory response syndrome and sepsis, the lung is one of the primarily affected organs. Even though data exist regarding the beneficial effects of adenosine on neutrophil activation [6–9], reversal of pulmonary hypertension [2] and oedema formation [3,4] in the context of acute lung injury, the impact of adenosine and its receptor sub-type-specific agonists is still virtually unexplored. In particular, tissue depletion of energy-rich adenine nucleotides in endotoxaemia as demonstrated by Sayeed [1], who observed significant reductions of ATP (−38%), ADP (−24%) and AMP (−34%), deserves explanation. Therefore, we investigated the effect of adenosine A_1 and A_2 receptor agonists on LPS-induced lung injury as well as tissue energy balance in 18 isolated perfused and ventilated rabbit lungs.

Haemodynamic reactions of the isolated rabbit lungs were in line with our previous data [18], which showed increases of PAP and oedema formation after LPS challenge compared with non-LPS-treated lungs due to an overweighing of vasoconstrictive pro-inflammatory mediators [16,17]. Within 2 h, pulmonary hypertension up to 20.8 ± 2.9 mmHg (Fig. 1, $P < 0.01$) occurred. Thiel and colleagues demonstrated similar pulmonary haemodynamic results during endotoxaemia [2]. In their study, adenosine administration delayed the beginning and the extent of the increase in pulmonary vascular resistance. In addition, fluid demand in adenosine-infused endotoxaemic animals was lower than in controls, indicating a better preservation of vascular integrity. They concluded that the reduction of pulmonary vascular resistance by adenosine might primarily be responsible for the prevention of the decrease in cardiac output [2], which is essential in patients with septic shock. Inosine, another purine compound, which is an adenosine degradation product via the enzyme adenosine-desaminase, likewise reduced pulmonary, intestinal and hepatic PMN-induced damage and hyperpermeability [23].

In our study, we further characterized the contribution of the adenosine receptor sub-types adenosine A_1 and adenosine A_2 to the protective effects of adenosine on endotoxin-induced acute lung injury. While pretreatment with adenosine A_1 agonist CCPA merely decelerated the pressure increase, adenosine A_2 agonist CPCA completely suppressed pulmonary pressure reaction. Adenosine via the adenosine A_1 receptor sub-type leads to adenosine efflux via protein kinase C (PKC)-dependent inhibition of adenosine kinase activity [24]. The resulting increase in locally available adenosine might then contribute to endothelium-dependent and -independent pulmonary vasodilatation via adenosine A_{2A} and adenosine A_{2B} receptors on vascular smooth muscle and endothelial cells [25,26]. However, adenosine A_1 receptor sub-type activation has also been demonstrated to mediate at least in part the LPS-induced release of thromboxane A_2 and IL-6 from endothelial cells [27]. This explains why the adenosine A_1 agonist in our study only decelerated but did not prevent the pulmonary pressure response. This is in line with the findings of Neely and colleagues, who reported protection from endotoxin-induced lung injury by adenosine A_1 receptor antagonism [28]. In contrast, the adenosine A_2 agonist CPCA also stimulates adenosine A_{2A} receptors expressed on neutrophils. This leads to reduced recruitment [7], adhesion [8] and trans-endothelial migration [6] of neutrophils. Subsequent reduction of oxygen-radical production [9] and decreased generation of pro-inflammatory mediators, such as TNFα [29], thromboxane A_2 and leukotriene C_4-E_4, [5] might underlie the prevention of LPS-induced pulmonary hypertension in our study.

Complex interactions between adenosine and nitric oxide synthesis in regulating regional vascular resistance have been postulated [26,30]. Corresponding to others, who found increases of iNOS not until 5 h of endotoxaemia [31], we found no major changes in NO_2/NO_3 levels within the observation period of up to 3 h [32], implying no relevant contribution of inducible NO synthase to pulmonary vasoregulation in the present model.

In the present study, both adenosine A_1 and adenosine A_2 agonist prevented LPS-induced oedema formation (Fig. 2). These data are in line with previous findings that adenosine reduces oedema development in the lung [2,33] and specific adenosine A_{2A} receptor activation reduced the wet-to-dry ratio in a model of lung transplantation [34]. In cell culture experiments adenosine lowered basal endothelial cell permeability [35] and blunted the activation of Rho A, which is intimately linked to cytoskeletal reorganization and regulation of endothelial barrier [36].

A major potential mechanism of pulmonary protection by adenosine via either the adenosine A_1 or adenosine A_2 receptor sub-type is amelioration of cellular damage induced by reactive oxygen species (ROS). Both systemic inflammation induced by endotoxin and ischaemia/reperfusion injury lead to oxidative stress. Superoxide anions are generated in large amounts by neutrophils during respiratory burst [37,38]. In endothelial cells, ROS are generated by inflammatory activation with TNFα [39] and engagement of endothelial cell adhesion molecules [40]. In this context, conversion of endothelial cell xanthine dehydrogenase to xanthine oxidase by neutrophil adhesion via a CD11a/CD18-dependent mechanism is of special interest [41]. Both ROS and neutrophil adhesion profoundly impair endothelial barrier function [42,43]. While both adenosine A_1 and adenosine A_{2A} receptors have been demonstrated to be protective in several models of oxidative stress, the underlying mechanisms differ. Endothelial cell monolayers exposed to xanthine/xanthine oxidase were protected from barrier dysfunction by an adenosine A_1 agonist [35] despite the known inhibitory effect of adenosine A_1 receptor activation on AC [44]. Adenosine A_1 receptor activation has been shown to induce preconditioning and protect myocardium [45,46] and other tissues [47] from ischaemic injury by increasing mitochondrial $K_{(ATP)}$ channel activity in a PKC-dependent manner [45,46]. A main feature of adenosine A_2 receptor stimulation is reduced neutrophil activation and adherence [4,6,29,34], resulting in diminished levels of oxidative stress and proinflammatory mediators.

In parallel with the reduction in pulmonary hypertension and the protective effects of adenosine A_1 and adenosine A_2 agonists on development of pulmonary oedema, we observed preserved tissue concentrations of energy-rich phosphates compared with isolated lungs during plain endotoxaemia. We suggest that adenosine A_1 and adenosine A_2 receptor activation leads to reduced levels of oxidative stress thereby diminishing energy-exhausting DNA repair and preserving tissue energy content. This hypothesis is supported by the maintenance of tissue NAD content in the adenosine receptor agonist treated lungs. Increased levels of oxidative stress are well recognized to impair cellular energy state. DNA strand breaks induced by ROS induce DNA-repair by the enzyme poly(ADP) ribose polymerase (PARP) under consumption of ATP and NAD [48]. Depletion of pulmonary and other tissues of energy-rich phosphates by LPS has previously been demonstrated [49]. Depletion of cellular ATP levels does not occur *per se* in the isolated lung, which keeps its ATP levels constant over at least 2 h [50].

The decreased CrP levels (Fig. 6) in the absence of adenosine agonists indicate that ATP supply does not meet the metabolic demand. Hence, the need exists to break down the energy storage form CrP [15] for maintenance of ATP levels. Decrease in the lung ATP contents during endotoxaemia was associated with impaired active Na^+ transport, being in part responsible for increases in pulmonary fluid volume [51] further enhancing pulmonary barrier dysfunction and oedema development.

The tissue content of high-energy phosphates has been determined in a lung homogenate by HPLC analysis. Therefore, the measures of adenosine and guanosine phosphates do not exactly represent the cellular energy state, because these phosphates could also be localized in the extracellular fluid. In this case, they are possibly not available for the intracellular energy metabolism. Further, differences in absolute tissue concentrations of nucleotides between the adenosine receptor agonist groups and the LPS control were significantly confounded by pulmonary oedema formation and subsequent dilution in the plain LPS group. Including absolute lung weight at the end of the observation period into co-variate analysis reduced the effect size of selective adenosine agonists to P levels of 0.08 and 0.06 for ATP and ADP, respectively. However, the calculations of ATP/ADP ratio and adenylate energy charge (Fig. 4) are robust with respect to oedema dilution and, thus, reflect significant preservation of tissue energy content by selective adenosine receptor agonists.

These improvements of pulmonary tissue energy content were paralleled by the observation that adenosine A_1 and adenosine A_2 agonists increased pulmonary cellular outward flux of adenosine. This might be due to competitive binding of adenosine agonists leading to increased non-receptor-bound free concentrations of adenosine. However, in the case of the adenosine A_1 agonist, there might be an additional mechanism as adenosine A_1 receptor activation inhibited adenosine kinase and led to enhanced cellular efflux of adenosine [24].

The clinical observation that high adenosine plasma concentrations could serve as a negative prognostic index for outcome in patients with septic shock [52] must be interpreted carefully [53]. Even considering adenosine as a degradation product of high-energy substrates, higher levels of this nucleoside might merely reflect an elevated energy turnover of the organism. To clearly state the role of adenosine as a prognostic marker in septic shock the knowledge of co-existing levels of both immediate energy donors, e.g. ATP and of storage forms of high-energy phosphates, such as CrP, is essential.

As other studies in this field [2,3], this work has the shortcoming of prophylactic pretreatment. Beneficial effects of adenosine, however, have also been demonstrated in therapeutic settings [11]. Future experiments subjected to treatment with adenosine A_1 and adenosine A_2 agonists in a post LPS-treatment setting will be helpful to evaluate the impact of these compounds as a therapeutic tool.

Taken together, failure of endothelial barrier function in the lung during endotoxaemia and depletion of energy-rich phosphates is mediated by a multitude of mechanisms. This work demonstrates the protective effects of adenosine A_1 and adenosine A_2 agonists on tissue energy content and vascular integrity after LPS-dependent breakdown of high-energy phosphate supply in the pulmonary vasculature. Thus, intervention into adenosine metabolism and adenosine dependent signaling pathways might fit into concepts of well-directed immuno-modulatory therapeutic strategies in acute inflammation.

Acknowledgements

This study was in part granted by the MedDrive programme of the Medical Faculty Carl Gustav Carus, Dresden, the Deutsche Forschungsgemeinschaft (DFG, KO1814/2-1), and the Research Fund of the Faculty of Clinical Medicine Mannheim, University of Heidelberg. The authors thank Dr Renate Urbaschek, Birgit Kaschta and Sylvia Thuy (Institute of Medical Microbiology and Hygiene), Kerstin Salomon, Jutta Schulte, Angelika Tapper and Monika Lehmer (Institute of Anesthesiology) and Jutta Christophel (Center of Medical Research, University Hospital of Mannheim, Germany) for excellent technical assistance. This study was presented at the Euroanaesthesia Congress in Glasgow, 31 May–3 June 2003.

References

1. Sayeed MM, Murthy PN. Adenine nucleotide and lactate metabolism in the lung in endotoxin shock. *Circ Shock* 1981; **8**: 657–666.
2. Thiel M, Kreimeier U, Holzer K, Moritz S, Peter K, Messmer K. Effects of adenosine on cardiopulmonary functions and oxygen-derived variables during endotoxemia. *Crit Care Med* 1998; **26**: 322–337.
3. Kutzsche S, Lyberg T, Bjertnaes LJ. Effects of adenosine on extravascular lung water content in endotoxemic pigs. *Crit Care Med* 2001; **29**: 2371–2376.
4. Adkins WK, Barnard JW, Moore TM, Allison RC, Prasad VR, Taylor AE. Adenosine prevents PMA-lung injury via an A_2 receptor mechanism. *J Appl Physiol* 1993; **74**: 982–988.
5. Neely CF, Keith IM. A_1 adenosine receptor antagonists block ischemia-reperfusion injury of the lung. *Am J Physiol* 1995; **268**: L1036–L1046.
6. Wakai A, Wang JH, Winter DC, Street JT, O'Sullivan RG, Redmond HP. Adenosine inhibits neutrophil vascular endothelial growth factor release and transendothelial migration via A2B receptor activation. *Shock* 2000; **15**: 297–301.
7. Cronstein BN. Adenosine, an endogenous anti-inflammatory agent. *J Appl Physiol* 1994; **76**: 5–13.
8. Thiel M, Chambers D, Chouker A, Bardenheuer HJ, Arfors K, Peter K. Effects of adenosine on the expression of β2-integrins and shedding of L-selectin of human polymorphonuclear leukocytes. *J Leukoc Biol* 1996; **59**: 671–686.
9. Thiel M, Bardenheuer H. Regulation of oxygen radical production of human polymorphonuclear leukocytes by adenosine: the role of calcium. *Pflügers Arch* 1992; **420**: 522–528.
10. Ohta A, Sitkovsky M. Role of G-protein-coupled adenosine receptors in downregulation of inflammation and protection from tissue damage. *Nature* 2001; **414**: 916–920.
11. Motew SJ, Mourelatos MG, Miller RN, Ferguson JL, Law WR. Evidence that adenosine contributes to maintenance of hepatosplanchnic blood flow during peritoneal sepsis in rats. *Shock* 1997; **7**: 439–446.
12. Weigand MA, Michel A, Eckstein HH, Martin E, Bardenheuer HJ. Adenosine: a sensitive indicator of cerebral ischemia during carotid endarterectomy. *Anesthesiology* 1999; **91**: 414–421.
13. Schmidt H, Weigand MA, Schmidt W, Plaschke K, Martin E, Bardenheuer HJ. Effect of dopexamine on intestinal tissue concentrations of high-energy phosphates and intestinal release of purine compounds in endotoxemic rats. *Crit Care Med* 2000; **28**: 1979–1984.
14. Deitch EA, Specian RD, Berg RD. Endotoxin induced bacterial translocation and mucosal permeability: role of xanthine oxidase, complement activation and macrophage products. *Crit Care Med* 1991; **19**: 785–791.
15. Conley KE, Kemper WF, Crowther GJ. Limits to sustainable muscle performance: interaction between glycolysis and oxidative phosphorylation. *J Exp Biol* 2001; **204**: 3189–3194.
16. Heller AR, Fiedler F, Braun P, Stehr SN, Bodeker H, Koch T. Clusterin protects the lung from leukocyte-induced injury. *Shock* 2003; **20**: 166–170.
17. Seeger W, Walmrath D, Grimminger F *et al*. Adult respiratory distress syndrome: model systems using isolated perfused rabbit lungs. *Methods Enzymol* 1994; **233**: 549–584.
18. Schmeck J, Heller A, Groschler A *et al*. Impact of endothelin-1 in endotoxin-induced pulmonary vascular reactions. *Crit Care Med* 2000; **28**: 2851–2857.
19. Behre G, Schedel I, Nentwig B, Wormann B, Essink M, Hiddemann W. Endotoxin concentration in neutropenic patients with suspected Gram-negative sepsis. *Antimicrob Agents Chemother* 1992; **36**: 2139–2146.
20. Watling KJ, ed. *The RBI Handbook of Receptor Classification and Signal Transduction*, 3rd edn. Natick MA/USA: Research Biochemicals International, 1998: 1–188.
21. Bardenheuer HJ, Fabry A, Höfling B, Peter K. Adenosine: a sensitive marker of myocardial ischaemia in man. *Cardiovasc Res* 1994; **28**: 656–662.

22. Lehninger AL. Oxidative phosphorylation, mitochondrial structure and the compartmentalization of respiratory metabolism. In: Lehninger AL, ed. *Biochemistry.* New York: Worth, 1975: 538–551.
23. Garcia Soriano F, Liaudet L, Marton A *et al.* Inosine improves gut permeability and vascular reactivity in endotoxic shock. *Crit Care Med* 2001; **29**: 703–708.
24. Sinclair CJ, Shepel PN, Geiger JD, Parkinson FE. Stimulation of nucleoside efflux and inhibition of adenosine kinase by A_1 adenosine receptor activation. *Biochem Pharmacol* 2000; **59**: 477–483.
25. McCormack DG, Clarke B, Barnes PJ. Characterization of adenosine receptors in human pulmonary arteries. *Am J Phyisol* 1989; **256**: H41–H56.
26. Olanrewaju HA, Mustafa SJ. Adenosine A(2A) and A(2B) receptors mediate nitric oxide production in coronary artery endothelial cells. *Gen Pharmacol* 2000; **35**: 171–177.
27. Wilson CN, Batra VK. Lipopolysaccharid binds to and activates A_1 adenosine receptors on human pulmonary artery endothelial cells. *J Endotoxin Res* 2002; **8**: 263–271.
28. Neely CJ, Jin J, Keith IM. A_1-adenosine receptor antagonists block endotoxin-induced lung injury. *Am J Physiol* 1997; **272**: L353–L361.
29. Thiel M, Chouker A. Acting via A_2 receptors, adenosine inhibits the production of tumor necrosis factor-alpha of endotoxin-stimulated human polymorphonuclear leukocytes. *J Lab Clin Med* 1995; **126**: 275–282.
30. Sam II AD, Sharma AC, Rice AN, Ferguson JL, Law WR. Adenosine and nitric oxide regulate regional vascular resistance via interdependent and independent mechanisms during sepsis. *Crit Care Med* 2000; **28**: 1931–1939.
31. Pastor CM, Hadengue A, Nussler AK. Minor involvement of nitric oxide during chronic endotoxemia in anesthetized pigs. *Am J Physiol Gastrointest Liver Physiol* 2000; **278**: G416–G424.
32. Heller A, Schmeck J, Heller S *et al.* Endothelin-1 impairs neutrophil respiratory burst and elimination of *Escherichia coli* in rabbits. *Crit Care Med* 2000; **28**: 1515–1521.
33. Jolin A, Myklebust R, Olsen R, Bjertnaes LJ. Adenosine protects ultrastructure of isolated rat lungs against fat emulsion injury. *Acta Anaesthesiol Scand* 1994; **38**: 75–81.
34. Reece TB, Ellman PI, Maxey TS *et al.* Adenosine A_2 receptor activation reduces inflammation and preserves pulmonary function in an *in vivo* model of lung transplantation. *J Thorac Surg* 2005; **129**: 1137–1143.
35. Richard LF, Dahms TE, Webster RO. Adenosine prevents permeability increase in oxidant-injured endothelial monolayers. *Am J Physiol* 1998; **274**: 35–42.
36. Harrington EO, Newton J, Morin N, Rounds S. Barrier dysfunction and Rho A activation are blunted by homocysteine and adenosine in pulmonary endothelium. *Am J Physiol Lung Cell Mol Physiol* 2004; **287**: L1091–L1097.
37. Lum H, Gibbs L, Lai L, Malik AB. CD18 integrin-dependent endothelial injury: effects of osponized zymosan and phorbol ester activation. *J Leukoc Biol* 1994; **55**: 58–63.
38. Tauber AI, Brettler DB, Kennington EA, Blumberg PM. Relation of human neutrophil phorbol ester receptor occupancy and NADPH-oxidase activity. *Blood* 1982; **60**: 333–339.
39. Corda S, Laplace C, Vicaut E, Duranteau J. Rapid reactive oxygen species production by mitochondria in endothelial cells exposed to tumor necrosis factor-α is mediated by ceramide. *Am J Respir Cell Mol Biol* 2001; **24**: 762–768.
40. Wang Q, Doerschuk CM. Neutrophil-induced changes in the biomechanical properties of endothelial cells: roles of ICAM-1 and adhesion molecules. *J Immunol* 2000; **164**: 6487–6494.
41. Wakabayashi Y, Fujita H, Morita I, Kawaguchi H, Murota S. Conversion of xanthine dehydrogenase to xanthine oxidase in bovine carotid artery endothelial cells induced by activated neutrophils: involvement of adhesion molecules. *Biochim Biophys Acta* 1995; **1265**: 103–109.
42. Lum H, Roebuck KA. Oxidant stress and endothelial cell dysfunction. *Am J Physiol* 2001; **280**: C719–C741.
43. Del Maschio A, Zanetti A, Corada M *et al.* Polymorphonuclear leukocyte adhesion triggers the disorganization of endothelial cell-to-cell adherens junctions. *J Cell Biol* 1996; **135**: 497–510.
44. Daly JW. Adenosine receptors: targets for future drugs. *J Med Chem* 1982; **25**: 197–207.
45. Sato T, Sasaki N, O'Rourke B, Marban E. Adenosine primes the opening of mitochondrial ATP-sensitive potassium channels: a key step in ischemic preconditioning? *Circulation* 2000; **102**: 800–805.
46. Narayan P, Mentzer Jr RM, Lasley RD. Adenosine A_1 receptor activation reduces reactive oxygen species and attenuates stunning in ventricular myocytes. *J Mol Cell Cardiol* 2001; **33**: 121–129.
47. Almeida CG, de Mendoca A, Cunha RA, Ribeiro JA. Adenosine promotes neuronal recovery from reactive oxygen species induced lesion in rat hippocampal slices. *Neurosci Lett* 2003; **339**: 127–130.
48. Bürkle A. Physiology and pathophysiology of poly(ADP-ribosyl)ation. *Bioessays* 2001; **23**: 795–806.
49. Holman RG, Maier RV. Oxidant-induced endothelial leak correlates with decreased cellular energy levels. *Am Rev Respir Dis* 1990; **141**: 134–140.
50. Rhoades RA. Isolated perfused lung preparation for studying altered gaseous environments. *Environ Health Perspect* 1984; **56**: 43–50.
51. Sayeed MM. Pulmonary cellular dysfunction in endotoxin shock: metabolic and transport derangements. *Circ Shock* 1982; **9**: 335–355.
52. Martin C, Leone M, Viviand X, Ayem ML, Guieu R. High adenosine plasma concentration as a prognostic index for outcome in patients with septic shock. *Crit Care Med* 2000; **28**: 3198–3202.
53. Gutierrez G. Sepsis, cellular energy metabolism, and tissue adenosine triphosphate concentration. *Crit Care Med* 2000; **28**: 2664–2665.