Exogenous adrenomedullin prevents and reverses hypodynamic circulation and pulmonary hypertension in ovine endotoxaemia

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Background. Hypodynamic septic shock is associated with a poor prognosis. The present randomized-controlled laboratory experiment was designed to test the hypothesis that the vasodilatory peptide hormone adrenomedullin (ADM) is a useful agent to prevent and reverse the development of hypodynamic circulation in ovine endotoxaemia.

Methods. Twenty-four healthy ewes were chronically instrumented for haemodynamic monitoring and randomly allocated to either the control, treatment, or prophylaxis group (n=8 each). After a baseline (BL) measurement in the healthy state, all sheep were subjected to a continuous endotoxin infusion started at 10 ng kg⁻¹ min⁻¹ and doubled every hour six times. After 4 h of endotoxin challenge, the treatment group received ADM (50 ng kg⁻¹ min⁻¹) for the remaining 3 h of the experiment. The prophylaxis group received a simultaneous infusion of endotoxin and ADM (50 ng kg⁻¹ min⁻¹) from the beginning to the end of the 7 h intervention period.

Results. In the control and treatment groups, the ewes exhibited a hypodynamic circulation at 4 h (>20% reduction in cardiac index, both P<0.01 vs BL). Endotoxin also increased mean pulmonary arterial pressure (MPAP) and arterial lactate concentrations. Prophylactic infusion of ADM prevented the occurrence of pulmonary hypertension and hypodynamic circulation and thereby blunted the increase in arterial lactate concentrations. In the treatment group, ADM administration increased CI (P<0.001) and reduced both MPAP (P=0.023) and arterial lactate concentrations (P<0.001 each at 7 h) when compared with the control group.

Conclusions. This study demonstrates that exogenous ADM prevents and reverses hypodynamic circulation, attenuates pulmonary hypertension, and limits lactic acidosis in ovine endotoxaemia.

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Adrenomedullin (ADM) is a 52 amino acid endogenous peptide hormone that was isolated from human pheochromocytoma cells by Kitamura and colleagues in 1993. Recent publications reveal that endogenous ADM is critically involved in endothelial protection and immunologic homeostasis in healthy subjects. Wang and colleagues reported that ADM is an essential mediator of hyperdynamic circulation in septic rats. In this context, it is noteworthy that rats treated with agents blocking the ADM system primarily develop hypodynamic septic shock. Recent studies indicate that exogenous ADM supplementation reduces mortality from septic shock and multiple organ failure (MOF) in rats. However, the role of ADM in the prevention and treatment of hypodynamic septic shock has only been investigated in small rodents. The hypothesis of the present study was that a continuous infusion of exogenous ADM is capable of preventing and treating hypodynamic shock in a clinically relevant model of ovine endotoxaemia. Therefore, the purpose of this study was to investigate whether exogenous ADM prevents and reverses the development of hypodynamic circulation in endotoxaemic sheep. Since ADM has been reported to reduce pulmonary vascular tone in patients with pulmonary arterial hypertension, an additional objective was to determine if and how exogenous ADM infusion impacts on the pulmonary circulation in the presence of hypodynamic endotoxaemic shock.

Methods

Animals

With approval of the Local Animal Research Committee, 24 healthy adult ewes [average weight 36.9 (1.1) kg] were chronically instrumented to determine cardiopulmonary haemodynamics and global oxygen transport using an established protocol.

Animal preparation

Induction of anaesthesia was performed by i.m. injection of S-ketamine (Ketanest 50, 10 mg kg\(^{-1}\), Parke-Davis, Berlin, Freiburg, Germany) and xylazine 2% (Xylazin, 0.15 mg kg\(^{-1}\), CEVA Tiergesundheit GmbH, Düsseldorf, Germany). After catheterization of a peripheral vein, anaesthesia was maintained using a continuous i.v. infusion of propofol (Disoprivan, 4–6 mg kg\(^{-1}\) h\(^{-1}\), AstraZeneca, Schwetzingen, Germany). The ewes remained unconscious but spontaneously breathing for the entire instrumentation period.

An indwelling pulmonary artery catheter was inserted via the right jugular vein through an introducer sheath (8.5 Fr. Catheter Introducer Set, pvb Medizintechnik GmbH, Kirchseeon, Germany; 7.5 Fr. Edwards Swan Ganz, Edwards Critical Care Division, Irvine, CA, USA). In addition, the sheep were instrumented with a left femoral arterial catheter (18-gauge Leader Cath; Vygon, Aachen, Germany) and a Foley catheter (Porgès S.A., Le Plessis Robinson-Cedex, France) to monitor urine output. Ceftriaxone (1 g ceftriaxone i.v.; Rocephin; Hoffmann-La Roche AG, Grenzach-Wyhlen, Germany) was administered for post-surgical infection prophylaxis.

Instrumentation was followed by a 24 h period of recovery. To prevent postoperative dehydration, all sheep received a continuous i.v. infusion of lactated Ringer’s solution (2 ml kg\(^{-1}\) h\(^{-1}\)).

Measurement equipment and determined variables

After instrumentation, intravascular catheters were connected to a physiological recorder (Hellige Servomed, Hellige, Freiburg, Germany) via pressure transducers (DTX pressure transducer, Ohmeda, Erlangen, Germany). Haemodynamic monitoring included mean arterial pressure (MAP), mean pulmonary arterial pressure (MPAP), central venous pressure (CVP), and pulmonary artery occlusion pressure (PAOP). Heart rate (HR) was determined by calculating the mean frequency of arterial pressure curve peaks. The thermodilution technique (9520A cardiac output computer; Edward Lifescience, Irvine, CA, USA) was used to measure cardiac output (CO) after threefold central venous injection of 10 ml of saline solution (0.9%) at a temperature of 2–5°C. CI, systemic vascular resistance index (SVRI), pulmonary vascular resistance index (PVRI), stroke volume index (SVI), and left and right ventricular stroke work indices (LVSWI, RVSWI) were determined using standard equations. Core body temperature (\(T\)) was continuously measured using the thermistor positioned at the tip of the pulmonary artery catheter.

Arterial and mixed venous blood samples (0.5 ml each) were collected in heparinized tubes designed to determine blood gases (Sarstedt Nümbrecht, Germany), \(pH\) and partial pressures of \(O_2\) and \(CO_2\) (\(P_{O_2}, P_{CO_2}\)) were determined using an ABL 725 blood gas analyzer with SAT 100 calibration (Radiometer Copenhagen, Denmark). In addition, haemoglobin concentration (Hb), arterial and mixed-venous oxygen saturation (\(S_{AO_2}, S_{vO_2}\)), and arterial lactate concentrations were assessed. Standard bicarbonate (HCO\(_3\)) base excess (BE) were calculated from \(P_{CO_2}\) and \(pH\). DO\(_2\)-I, oxygen consumption index (VO\(_2\)-I), and oxygen extraction rate (O\(_2\)-ER) were determined using the standard formulae.

Experimental protocol

Inclusion criteria for the present study were an initial HR <100 beats min\(^{-1}\), core body temperature \(\leq 39.8^\circ\)C, MPAP <20 mm Hg, and arterial lactate \(\leq 1\) mmol litre\(^{-1}\).
During the experimental protocol, all ewes were spontaneously breathing and studied in a conscious state. Animals were housed in metabolic cages with free access to water and food.

After a baseline (BL) measurement in the healthy state, the ewes were randomized to treatment, prophylaxis, or control groups (n=8 each). All three groups received a continuous infusion of Salmonella typhosa endotoxin (Sigma Chemicals, Deisenhofen, Germany, Catalogue # L6386-100 mg), which was started at a rate of 10 ng kg⁻¹ min⁻¹. Thereafter, the endotoxin dose was doubled every hour during the 7 h intervention period (highest rate 640 ng kg⁻¹ min⁻¹). In dose–response studies, we previously determined that this approach reliably results in a hypodynamic circulation within 4 h of endotoxin infusion. In this context, hypodynamic circulation was defined as a MAP reduction in CI of more than 20% when compared with BL. Hypodynamic shock was defined by a MAP<65 mm Hg in the presence of hypodynamic circulation.

At the same time as the endotoxin infusion was started, lactated Ringer’s solution was adjusted to maintain PAOP and CVP at BL values ±3 mm Hg.

In the prophylaxis group, an i.v. infusion of ADM (human ADM; 50 ng kg⁻¹ min⁻¹; Bachem AG, Bubendorf, Switzerland, Catalogue # H-2932.1000) was started at a rate of 10 ng kg⁻¹ min⁻¹. Thereafter, the endotoxin dose was doubled every hour during the 7 h intervention period (highest rate 640 ng kg⁻¹ min⁻¹). Haemodynamic variables and oxygen transport data were analysed at BL and every hour for a total of 7 h (1–7 h).

At the end of the experiment, the ewes were deeply anaesthetized with propofol (4 mg kg⁻¹) and killed with a lethal dose of 100 ml of potassium chloride solution (7.45%).

Statistical analysis

Data are expressed as mean (SEM). Sigma Stat 3.10 software (SPSS, Chicago, IL, USA) was used for statistical analysis. After confirming normal distribution of all variables (Kolmogorov–Smirnov test), differences within and between the groups were analysed using a two-way analysis of variance for repeated measurements (RM-ANOVA).

After confirming significant group differences over time, appropriate post hoc comparisons (Student–Newman–Keuls) were performed. For all statistical tests, an α-error probability of P<0.05 was regarded as statistically significant.

Results

All 24 ewes survived the 7 h intervention period. There were no statistical differences in haemodynamic and global oxygen transport variables between the groups at randomization.

In all groups, core body temperature increased, whereas haematocrit, CVP, and PAOP remained constant throughout the entire experiment (Table 1).

Effects of endotoxin infusion

Endotoxin infusion altered MAP, CI, and DO₂I in a dose–dependent manner (Fig. 2). In the control group, the ewes exhibited a hypodynamic circulation at 4 h [CI 4.7 (0.2) vs 6.3 (0.2) litre min⁻¹ m⁻², P=0.007 vs BL] and hypodynamic shock at 7 h [CI 4.6 (0.3) vs 6.3 (0.2) litre min⁻¹ m⁻², MAP 63 (3) vs 98 (2) mm Hg, each P<0.001 vs BL]. Myocardial inotropy was suppressed, as reflected by decreases in LVSWI and SVI (both P<0.001 vs BL at 4 and 7 h; Table 1). These alterations of the systemic circulation were accompanied by significant increases in MPAP and PVRI (both P<0.001 at 4 and 7 h vs BL; Fig. 2). In addition to a decrease in DO₂I (P=0.038 at 7 h vs BL; Fig. 3), the ewes were characterized by significant increases in arterial lactate concentrations [3.6 (0.4) vs 0.7 (0.1) mmol litre⁻¹, P<0.001 at 7 h vs BL; Fig. 3].

![Fig 1](https://example.com/diagram.png) Schematic diagram of the study design. ADM, adrenomedullin; BL, baseline.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>BL</th>
<th>4 h</th>
<th>7 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=8)</td>
<td>94 (7)</td>
<td>44 (4)</td>
<td>25 (1)</td>
<td></td>
</tr>
<tr>
<td>Prophylaxis (n=8)</td>
<td>92 (7)</td>
<td>68 (5)*</td>
<td>46 (3)*</td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>89 (5)</td>
<td>52 (6)</td>
<td>46 (2)*</td>
<td></td>
</tr>
<tr>
<td>RVSWI (g m⁻¹ m⁻²)</td>
<td>95 (1)</td>
<td>63 (2)</td>
<td>46 (1)*</td>
<td></td>
</tr>
<tr>
<td>Prophylaxis</td>
<td>16 (2)</td>
<td>20 (1)*</td>
<td>16 (1)*</td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>14 (1)</td>
<td>16 (1)</td>
<td>17 (1)*</td>
<td></td>
</tr>
<tr>
<td>SVI (ml beat⁻¹ m⁻²)</td>
<td>66 (1)</td>
<td>75 (5)*</td>
<td>70 (2)*</td>
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</tr>
<tr>
<td>Treatment</td>
<td>63 (2)</td>
<td>45 (2)</td>
<td>68 (4)*</td>
<td></td>
</tr>
<tr>
<td>CVP (mm Hg)</td>
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<td>6 (1)</td>
<td>6 (1)</td>
<td></td>
</tr>
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</tr>
<tr>
<td>Treatment</td>
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<td>4 (1)</td>
<td>4 (1)</td>
<td></td>
</tr>
<tr>
<td>PAOP (mm Hg)</td>
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<td>13 (1)</td>
<td>13 (1)</td>
<td></td>
</tr>
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<td>12 (1)</td>
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<tr>
<td>Treatment</td>
<td>9 (1)</td>
<td>10 (1)</td>
<td>10 (1)</td>
<td></td>
</tr>
<tr>
<td>T (°C)</td>
<td>39.5 (0.1)</td>
<td>41.1 (0.3)</td>
<td>41.0 (0.2)</td>
<td></td>
</tr>
<tr>
<td>Prophylaxis</td>
<td>39.6 (0.1)</td>
<td>41.3 (0.2)</td>
<td>41.4 (0.2)</td>
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<tr>
<td>Treatment</td>
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<td>41.4 (0.1)</td>
<td>41.1 (0.2)</td>
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</tr>
</tbody>
</table>
Effects of prophylactic ADM infusion
The prophylaxis group exhibited a hypotensive-hyperdynamic circulation, characterized by an increase in CI [10.3 (0.5) vs 4.7 (0.2); \( P<0.001 \) vs control at 4 h] and decreases in MAP (\( P=0.016 \) vs control at 4 h) and SVRI (\( P<0.001 \) vs control at 4 and 7 h; Fig. 2). Notably, there was no statistical difference in MAP between the groups at the end of the observation period (\( P=0.481 \)). Myocardial inotropy was maintained by ADM infusion as reflected by higher LVSWI (\( P=0.002 \) and \( P<0.001 \) vs control at 4 and

Fig 2 Changes in systemic and pulmonary variables in endotoxaemic sheep treated with prophylactic or therapeutic adrenomedullin. BL, baseline; CI, cardiac index; HR, heart rate; MAP, mean arterial pressure; MPAP, mean pulmonary arterial pressure; PVRI, pulmonary vascular resistance index; SVRI, systemic vascular resistance index; 
* \( P<0.05 \) prophylaxis vs control; 
† \( P<0.05 \) treatment vs control.
Effects of therapeutic ADM infusion

There was no statistical difference in any variable between the control and the treatment groups during the initial 4 h of endotoxaemia (Figs 2 and 3, Table 1). Initiation of therapeutic ADM infusion (at 4 h) was followed by marked increases in CI, DO2I, LVSWI, and SVI and a reduction in SVRI (all $P<0.001$ vs control at 7 h). Notably, apart from an initial decrease in arterial pressure, there was no statistical difference in MAP ($P=0.726$) or HR ($P=0.144$) between the treatment and the control group (Fig. 2).

Therapeutic ADM infusion reduced MPAP ($P=0.023$ at 7 h) and PVRI ($P<0.001$ at 7 h) when compared with the untreated control group (Fig. 2). At the end of the observation period, all haemodynamic variables were similar in the treatment and the prophylaxis group.

Whereas DO2I increased in the treatment group, O$_2$-ER decreased ($P<0.001$ and $P=0.003$ vs control at 7 h; respectively). These changes in global oxygen transport were associated with a limited increase in arterial lactate concentrations when compared with the control group ($P<0.001$ at 7 h; Fig. 3).

Discussion

The present study was conducted to investigate the effects of therapeutic and prophylactic ADM administration on cardiopulmonary haemodynamics and global oxygen transport in endotoxaemic sheep. The key findings are the following:

- Prophylactic ADM infusion at 50 ng kg$^{-1}$ min$^{-1}$ prevents the development of hypodynamic shock and pulmonary hypertension and limits lactic acidosis in ovine endotoxaemia.
- Therapeutic infusion of ADM (50 ng kg$^{-1}$ min$^{-1}$) converts hypodynamic circulation into a hyperdynamic haemodynamic state, attenuates pulmonary hypertension, and limits arterial lactate concentrations to the same extent as prophylactic ADM administration.
- Although ADM is a potent vasodilator, it caused only a transient decrease in MAP in sheep allocated to the treatment group when compared with the control group. In addition, HR was not impaired in the treatment group beyond the changes caused by endotoxin itself.

The roles of endogenous and exogenous ADM have been extensively studied in rodent septic shock. In the latter model and in clinical septic shock, endogenous plasma ADM levels are elevated in response to systemic inflammation. Wang and colleagues demonstrated that endogenous ADM is an essential mediator of the hyperdynamic response in early sepsis, since septic rats pre-treated with anti-ADM antibodies primarily developed hypodynamic shock. However, the efficacy of endogenous and exogenous ADM is reduced in the late stage of septic...
which increases intracellular calcium (Ca$^{2+}$) in vascular endothelial cells, ADM activates phospholipase C, increasing CI and DO2I in healthy sheep and in sheep suffering from hyperdynamic endotoxaemia. Recent studies likewise demonstrated that exogenous ADM markedly improved survival and reduced tissue injury in septic and endotoxemic rats in a dose-dependent manner.

The purpose of the present study was to elucidate whether exogenous ADM is able to prevent and treat hypodynamic circulation in severe ovine endotoxaemia. In pilot projects, our research group demonstrated that a continuous infusion of 50 ng kg$^{-1}$ min$^{-1}$ ADM effectively increases CI and DO2I in healthy sheep and in sheep suffering from hyperdynamic endotoxaemia. In the present study, endotoxin administration impaired myocardial contractility, as reflected by marked decreases in LVSWI and SVI in the control group. Infusion of ADM restored BL values of the latter variables, thereby re-establishing hyperdynamic circulation, even when given after the onset of hypodynamic shock. Notably, in contrast to the prophylaxis group, there was no increase in HR or sustained arterial hypertension in the treatment group compared with the control group, despite an initial decrease in MAP. Therefore, it appears that potential side-effects of exogenous ADM (such as arterial hypertension and reflex tachycardia) are of limited importance in severely ill subjects. This may, in part, be explained by the improvement in myocardial contractility, as suggested by increases in LVSWI and SVI in response to ADM infusion. In this regard, it may be important that ADM has abundant binding sites on the ventricular myocardium and improves ventricular performance by both cyclic adenosine monophosphate (cAMP)-dependent and -independent mechanisms.

Another interesting finding of the present study is that exogenous ADM prevented and reversed endotoxin-induced pulmonary hypertension. Nagaya and colleagues demonstrated that i.v. and aerosolized ADM effectively reduce MPAP in patients with primary pulmonary arterial hypertension. However, the effects of ADM on sepsis-associated pulmonary hypertension have not yet been investigated in the clinical setting.

The mechanisms of the ADM-induced systemic and pulmonary vasodilatory effects include activation of the calcitonin receptor-like receptor (CRLR) and its associated receptor activity-modifying proteins (RAMP 1–3). The CRLR/RAMP complex stimulates synthesis of cAMP, which in turn induces relaxation of vascular smooth muscle cells via activation of protein kinase A. Conversely, several ADM-related effects are not mediated by CRLRs and appear to be independent of the cAMP system. In vascular endothelial cells, ADM activates phospholipase C, which increases intracellular calcium (Ca$^{2+}$) via the inositol-1,4,5-trisphosphate (IP$_3$) system. Increases in Ca$^{2+}$ concentrations in endothelial cells, in turn, directly stimulate NO production via endothelial NO synthase (eNOS). Endothelium-derived NO then activates the cyclic guanosyl monophosphate (cGMP) system in vascular smooth muscle cells, thereby mediating vasodilation.

In addition to its systemic and pulmonary vasodilatory properties, prophylactic and therapeutic ADM administration limited the increase in arterial lactate concentrations at the later stages of endotoxaemia in the present study. Since we did not investigate microcirculatory blood flow and mitochondrial function, the exact mechanisms of this beneficial effect remain to be determined in future studies. However, the markedly increased O$_2$-ER and decreased DO$_2$I in the control group suggest impaired tissue perfusion in untreated sheep as a key factor for systemic generation of lactic acid. This assumption is supported by a recent study of Gonzalez-Rey and colleagues, reporting decreased levels of arterial lactate, transaminases, and pro-inflammatory cytokines after gut ischaemia–reperfusion injury in rats treated with ADM/AMBP-1.

The current study has some limitations. First, we did not investigate specific variables of organ perfusion and function. Therefore, we can only draw conclusions about global haemodynamics and oxygen supply. Secondly, a prophylactic infusion of ADM before the onset of shock may be of limited value in the clinical setting. However, the present study clearly shows that therapeutic administration of ADM in hypodynamic sheep improves haemodynamics and oxygenation to similar endpoints of CI, MPAP, and arterial lactate concentrations when compared with the prophylaxis group. Thus, a therapeutic infusion of ADM appears to be equally effective as the prophylactic administration. Thirdly, we investigated the effects of human ADM in sheep. To our knowledge, the amino acid structure of ovine ADM has not yet been determined. Ovine ADM is not currently available. However, our study group and others have demonstrated that infusion of 50 ng kg$^{-1}$ min$^{-1}$ of human ADM results in similar haemodynamic and metabolic alterations in sheep and humans. We therefore decided to use human ADM at the specified dose. Finally, infusion of a vasodilator in hypotensive subjects may be associated with deteriorated perfusion pressure and reflex tachycardia. This in turn may impair the relationship between myocardial oxygen supply and demand. Notably, in the present study, therapeutic ADM administration was not linked to increases in HR or sustained reductions in MAP when compared with the untreated control group.

Conclusions

In conclusion, exogenous ADM (50 ng kg$^{-1}$ min$^{-1}$) was capable of preventing and treating hypodynamic circulation and pulmonary hypertension in endotoxemic ewes. These haemodynamic changes occurred in parallel with
reduced arterial lactate concentrations, suggesting an improvement in tissue perfusion and oxygenation. Despite systemic and pulmonary vasodilatatory properties of ADM, the marked increases in variables of myocardial contractility after therapeutic (not prophylactic) ADM administration were neither associated with tachycardia nor sustained arterial hypotension when compared with untreated controls. In view of the high mortality of patients with refractory hypodynamic septic shock states, randomized trials are warranted to investigate the role of ADM in the clinical setting of septic shock.

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