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Biochemical and Biophysical Research Communications 296 (2002) 134–138

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Hemodynamic effects of exogenous adrenomedullin in healthy and endotoxemic sheep

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Received 18 June 2002

Abstract

Adrenomedullin (AM) is a vasodilatory peptide hormone, playing a key role in the regulation of cardiovascular homeostasis. In view of the circulatory failure in sepsis, it is still debated as to whether the occurrence of vascular hyporeactivity against AM plays a causative or protective role. This study was designed as a prospective, controlled trial to elucidate the hemodynamic response following a titrating infusion of human AM in healthy and endotoxemic sheep. ANOVA demonstrated that AM infusion produced hypotension and tachycardia, and increased cardiac index in a dose-dependent manner, both in healthy and endotoxemic sheep. In addition, AM application reduced pulmonary vascular resistance index in ovine endotoxemia ($P = 0.02$). These findings confirm that AM produces a hyperdynamic circulation, in the presence and absence of systemic inflammation. Further, exogenous AM could possibly be a useful adjunct in the common setting of sepsis-associated pulmonary hypertension.

Keywords: Adrenomedullin; Circulation; Endotoxemia; Hemodynamics; Lung; Sepsis; Sheep; Shock

Despite significant progress in understanding the pathophysiology of sepsis and septic shock, these conditions continue to be the most causes of morbidity and mortality in intensive care units [1]. Determination of the mechanisms responsible for cardiovascular failure might enable a lowering in risk for cell and organ dysfunction. The current treatment regimen for septic patients consists of a targeted antimicrobial therapy, and a sufficient organ support. For the latter condition, fluid resuscitation and vasopressor agents play a pivotal role [2].

Recently, experimental studies identified adrenomedullin (AM), a 52-amino acid peptide hormone, as a key molecule in the regulation and preservation of cardiovascular homeostasis [3,4]. AM is a potent vasorelaxant produced by posttranslational splicing of proadrenomedullin together with proadrenomedullin N-terminal 20 peptide (PAMP), another hypotensive protein [5]. AM is synthetized by various tissues including the digestive organs, kidney, lung, liver, heart, and vasculature [6,7]. Following surgical and inflammatory stimuli, AM is liberated into the circulation [8,9], and is currently regarded as one of the most important counterregulatory mediators in human heart failure [10]. In addition, AM exhibits sustained elevation starting in the early phase of sepsis and plays a major role in initiating the hyperdynamic circulation [11]. As sepsis progresses, however, vascular hyporesponsiveness to AM occurs, and appears to produce the transition to the late moribund, hypodynamic phase [12]. An exogenous application of AM could therefore be a rationale to preserve the function of cardiovascular and other organ systems. Since AM possesses significant vasodilator and positive chronotropic properties [13], it could also be possible that a sole administration of AM does not improve but deteriorate the hemodynamic alterations found in hyperdynamic sepsis, e.g., hypotension and tachycardia. In view of the cardiovascular failure in sepsis, it has not been clarified as to whether the occurrence of vascular hyporeactivity against AM is a causative or protective effect.

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0006-291X/02/$ - see front matter
PII: S0006-291X(02)00821-5

Our objective was to elucidate whether AM has different effects on the cardiopulmonary circulation under physiological, healthy and pathological, septic conditions. Accordingly, this study was designed as a prospective, controlled laboratory experiment to compare the hemodynamic response following a continuous AM infusion in healthy and endotoxemic sheep.

Methods

After approval by the Government Animal Research Committee, seven adult ewes, weighing 38 ± 2 kg were instrumented for chronic study.

Animal preparation. After inducing anesthesia with intramuscular xylazine 2% (Xylazin, 0.15 mg kg^{-1}; CEVA Tiergesundheit GmbH, Düsseldorf, Germany) and ketamine (Ketanest 50, 15 mg kg^{-1}; Parke-Davis Berlin, Freiburg, Germany), the ewes were chronically instrumented with an indwelling pulmonary artery catheter positioned percutaneously through an introducer sheath via the right jugular vein (8.5 Fr. Catheter Introducer Set; pvb Medizintechnik GmbH, Kirchseeon, Germany; and 7.5 Fr. Edwards Swan Ganz; Edwards Critical Care Division, Irvine, CA). In addition, a femoral arterial catheter (18-gauge Leader Cath; Vygon, Aachen, Germany) was inserted into the left femoral artery. During the instrumentation, anesthesia was maintained with a continuous intravenous infusion of propofol (Disoprivan, 4–5 mg kg^{-1} h^{-1}; AstraZeneca, Schwetzigen, Germany).

After insertion of the pulmonary artery catheter, the ewes received a single shot infusion of Ceftriaxon (Rocephin; Hoffmann-La Roche AG, Grenzach-Wyhlen, Germany), and a continuous intravenous infusion of Ringer's lactate solution (2 ml kg^{-1} h^{-1}).

Following a 24-h period of recovery, catheters were connected to pressure transducers (DTX pressure transducer; Ohmeda Ltd. and Co. KG, Erlangen, Germany) and a physiological recorder (Hellige Servomed; Hellige Ltd., Freiburg, Germany), to monitor heart rate (HR), mean arterial pressure (MAP), mean pulmonary arterial pressure (MPAP), central venous pressure (CVP), and pulmonary capillary wedge pressure (PCWP). Cardiac output was estimated with the thermodilution technique, applying the Fick Principle (9520 A cardiac output computer; Edward Lifescience, Irvine, CA). Measurements were performed, using triplicate injections of 2–5 °C cold saline solution. Cardiac index (CI), systemic vascular resistance index (SVRI), and pulmonary vascular resistance index (PVRI) were calculated using standard equations.

All measurements were performed in awake and spontaneously breathing animals, which were housed and studied in metabolic cages with water and food ad libitum throughout the entire experiment.

Experimental protocol. Animals were included in the study if HR was <100 beats min^{-1} and MPAP was <25 mmHg. After a baseline measurement in the healthy state, the animals received a continuous infusion of human AM (1–52; BACHEM Biochemica GmbH, Heidelberg, Germany) at 10, 50, and 100 ng^{-1} kg^{-1} min^{-1}, each dose for 30 min. Thereafter, drug infusions were stopped.

Hemodynamics were obtained at baseline and every 30 min during the experiment.

After a 24-h period of recovery, the same sheep were subjected to a continuous infusion of salmonella typhosa endotoxin (10 ng kg^{-1} min^{-1}; Sigma Chemicals, Deisenhofen, Germany) for the next 25.5 h.

In the surviving animals ($n = 7$), cardiopulmonary variables were analysed after 24 h of a continuous endotoxin infusion. Then, AM was administered again, following the same protocol that was used in the healthy state.

At the end of the experiment, the ewes were anesthetized with a bolus infusion of propofol (4 mg kg^{-1}; Disoprivan) and killed with a lethal dose of 100 ml potassium chloride (7.45%).

Statistical analysis. Data are expressed as means ± SEM. For statistical analysis, Sigma Stat 2.03 software (SPSS, Chicago, IL, USA) was used. After testing for normal distribution (Kolmogorov–Smirinov), we performed a paired Student's t test to compare data between groups. To analyse the effects of the treatments, a two-way analysis of variance (ANOVA) for repeated measurements with appropriate post hoc comparisons (Student–Newman–Keuls) were performed. For all statistical tests, an error probability of $P < 0.05$ was regarded as significant.

Results

After 24 h of endotoxemia, all sheep ($n = 7$) exhibited a hypotensive–hyperdynamic circulation with a significant decrease in MAP and SVRI ($P < 0.001$; Fig. 1) and an increase in HR and CI ($P < 0.001$; Fig. 2).

In a dose-related manner, AM infusion decreased MAP and SVRI in healthy and endotoxemic sheep (Fig. 1). This was accompanied by a significant increase in HR

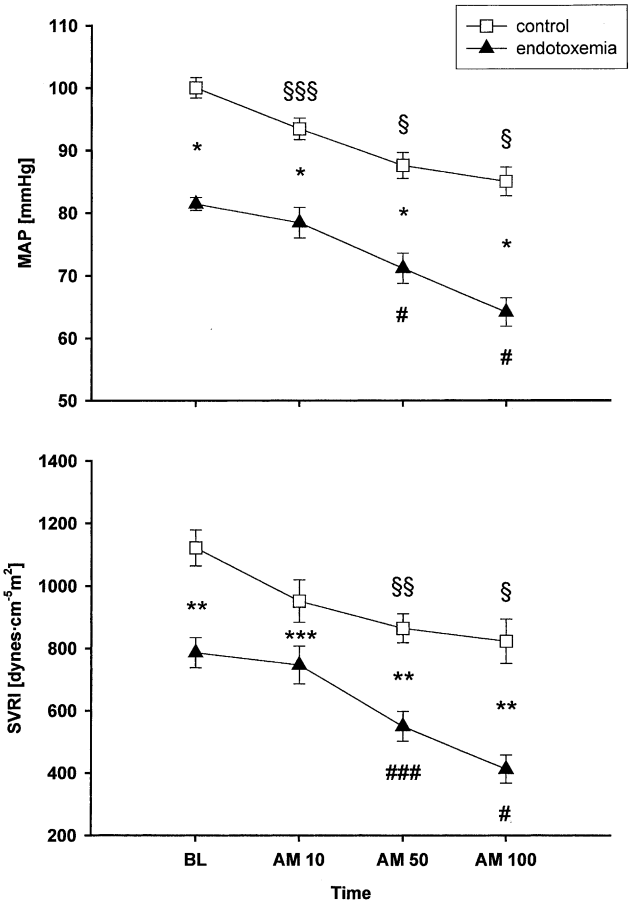

Fig. 1. Mean arterial pressure (MAP) and systemic vascular resistance index (SVRI) before (BL) and after a continuous adrenomedullin infusion at 10 (AM 10), 50 (AM 50), and 100 (AM 100) ng kg^{-1} min^{-1} in healthy and endotoxemic sheep. (□), healthy controls ($n = 7$); (▲), sheep after 24 h of endotoxemia ($n = 7$). $^{***}P < 0.05$, $^{**}P < 0.01$, $^{*}P < 0.001$ endotoxemia versus control; $^{###}P < 0.05$, $^{##}P < 0.01$, $^{#}P < 0.001$ versus BL in endotoxemia; $^{§§§}P < 0.05$, $^{§§}P < 0.01$, $^{§}P < 0.001$ versus BL in controls.

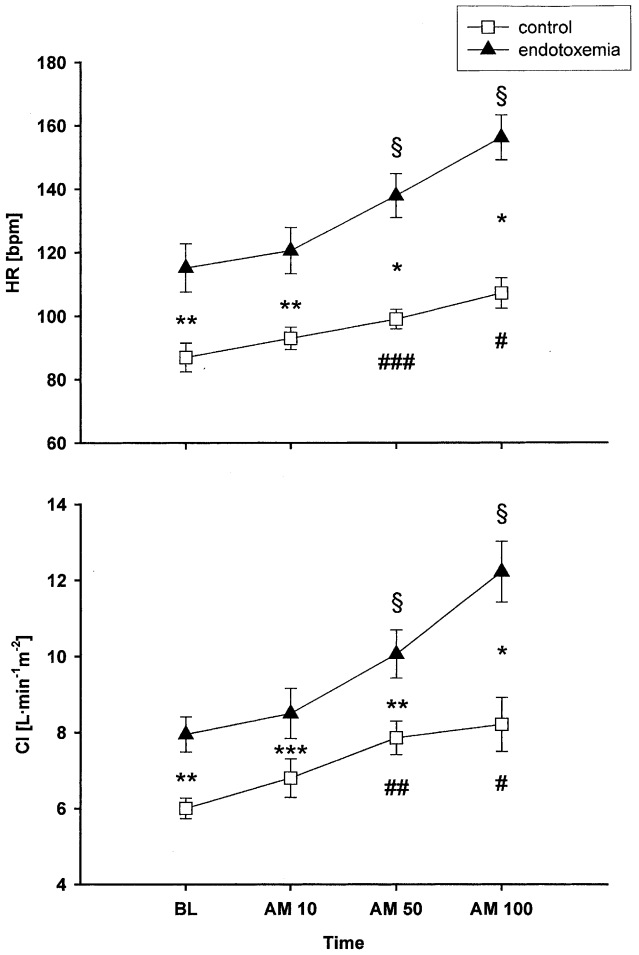

Fig. 2. Mean heart rate (HR) and cardiac index (CI) before (BL) and after a continuous adrenomedullin infusion at 10 (AM 10), 50 (AM 50), and 100 (AM 100) $ng\,kg^{-1}\,min^{-1}$ in healthy and endotoxemic sheep. (□), healthy controls ($n = 7$); (▲), sheep after 24 h of endotoxemia ($n = 7$). $^{***}P < 0.05$, $^{**}P < 0.01$, $^{*}P < 0.001$ endotoxemia versus control; $^{\#\#\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#}P < 0.001$ versus BL in endotoxemia; $^{\S\S\S}P < 0.05$, $^{\S\S}P < 0.01$, $^{\S}P < 0.001$ versus BL in controls.

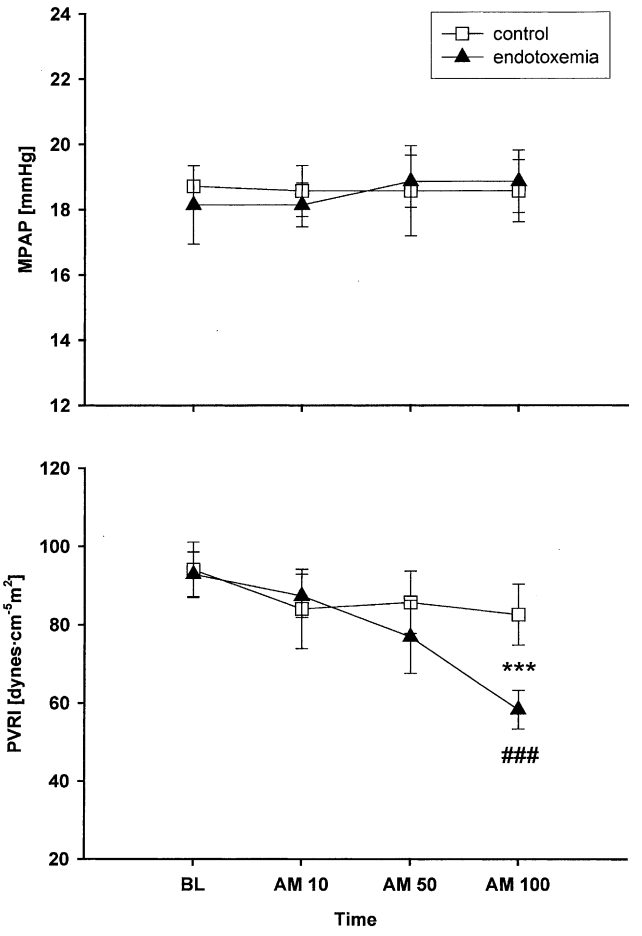

Fig. 3. Mean pulmonary arterial pressure (MPAP) and pulmonary vascular resistance index (PVRI) before (BL) and after a continuous adrenomedullin infusion at 10 (AM 10), 50 (AM 50), and 100 (AM 100) $ng\,kg^{-1}\,min^{-1}$ in healthy and endotoxemic sheep. (□), healthy controls ($n = 7$); (▲), sheep after 24 h of endotoxemia ($n = 7$). $^{***}P < 0.05$, $^{**}P < 0.01$, $^{*}P < 0.001$ endotoxemia versus control; $^{\#\#\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#}P < 0.001$ versus BL in endotoxemia; $^{\S\S\S}P < 0.05$, $^{\S\S}P < 0.01$, $^{\S}P < 0.001$ versus BL in controls.

and CI (Fig. 2). In endotoxemic but not in healthy sheep, AM ($100\,ng^{-1}\,kg^{-1}\,min^{-1}$) decreased PVRI ($P = 0.02$). No changes occurred for MPAP in both study groups before and after application of AM (Fig. 3).

Discussion

In the present study, we determined whether vascular responsiveness to exogenously administered AM is different under physiologic, healthy and pathologic, septic conditions. The major findings of our investigation are (1) a potent systemic vasodilatory effect of AM, which was accompanied by tachycardia and an increased CI, both in healthy and endotoxemic sheep, and (2) an AM-associated reduction in PVRI, exclusively during endotoxemia.

The findings of our study are supported by recent reports, demonstrating AM to play a pivotal role in producing hyperdynamic circulation in a rat model of polymicrobial sepsis [11,14]. However, the factors responsible for hemodynamic and cardiovascular alterations after the onset of sepsis are still not fully understood. In previous small-scale experimental studies on animals with hypodynamic septic shock hyporeactivity against AM occurred despite the fact that plasma AM levels were remarkably increased [12]. The authors of the same investigation concluded that the vascular hyporesponsiveness against endogenous AM could be responsible for circulatory collapse [12]. To our knowledge, this is the first study demonstrating that exogenous AM has a similar impact on cardiac performance in a healthy and a septic condition. We hypothesized that due to its vasorelaxant effect, AM application could possibly worsen the hypotensive–hyperdynamic circulation in

sepsis. In this regard, it is noteworthy, that in a dose-related manner, AM did not improve but augment hyperdynamic circulation found during endotoxemia. Thus, it remains uncertain, whether exogenous administration of AM in hyperdynamic sepsis is a useful or dangerous agent. However, it remains undetermined as to how AM affects the microcirculation. Since AM is ubiquitously expressed in the organs, playing a major role in the genesis and maintenance of the septic cascade, e.g., kidney, liver, and gut [6,7], it may also be possible that due to its vasodilatory effect in the capillary beds, organ damage can be mitigated or even avoided. Therefore, further studies are needed to address this issue.

Because it is still controversial as to whether AM plays a significant role under physiological conditions [15], we additionally studied the hemodynamic effects of AM at incrementing doses in healthy sheep. We demonstrated that exogenous AM actually regulates vascular tone. As in endotoxemia, AM infusion was accompanied by hypotension, tachycardia, and an increase in cardiac index. These findings, therefore, confirm that elevated AM levels produce hyperdynamic circulation, in the presence and absence of systemic inflammation.

To date, the effect of AM on the pulmonary circulation has not been studied in detail. Since lung tissue in general and pulmonary vasculature in particular produce a variety of vasoactive mediators including AM [16], we assumed that exogenous application of AM could also affect the pulmonary circulation. In endotoxemic, but not in healthy animals, AM reduced PVRI significantly. This decrease in PVRI was associated with a substantial increase in CI, whereas MPAP remained constant. In contrast to the decrease in SVRI, no significant decrease in PVRI was found in healthy sheep after application of AM. The different effect of AM on the systemic and the pulmonary vascular bed could be due to a heterogeneity of the AM receptors and the distribution of AM receptors within and between the vascular beds. In this regard, it has been demonstrated that AM transcripts and protein are presented in the lungs, and that endotoxin infusion per se augments AM gene expression [17,18]. In addition, these findings underline that AM plays a pivotal role, not only as a circulating hormone, but also as a local autocrine and/or paracrine mediator [19]. While endotoxemia usually results in pulmonary vasoconstriction [20,21], pretreatment with AM seems to prevent such vasoconstriction, as shown in the here presented study. From this point of view, AM could possibly be a useful adjunct in the treatment or prophylaxis of sepsis-associated pulmonary hypertension.

Taken together, our results indicate that application of synthetic human AM improves the pulmonary circulation in ovine endotoxemia, but augments the hemodynamic characteristics of hyperdynamic circulation in a dose-dependent manner. It therefore remains ambiguous whether upregulation of AM during sepsis really plays a protective role. Thus, further studies should verify that the AM-related vasodilation does not occur at the expense of compromised organ functions, for example as a result of excessive hypotension and tachycardia.

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