The Pivotal Role of Adrenomedullin in Producing Hyperdynamic Circulation During the Early Stage of Sepsis

Ping Wang, MD; Zheng F. Ba; William G. Cioffi, MD; Kirby I. Bland, MD; Irshad H. Chaudry, PhD

Background: Initial cardiovascular responses during sepsis are characterized by hyperdynamic circulation. Although studies have shown that a novel potent vasodilatory peptide, adrenomedullin (ADM), is up-regulated under such conditions, it remains unknown whether ADM is responsible for initiating the hyperdynamic response.

Objective: To determine whether increased ADM release during early sepsis plays any major role in producing hyperdynamic circulation.

Design, Intervention, and Main Outcome Measure: Synthetic rat ADM (8.5 µg/kg of body weight) was infused intravenously in normal rats for 15 minutes at a constant rate. Cardiac output, stroke volume, and microvascular blood flow in various organs were determined immediately as well as 30 minutes after ADM infusion. At 30 minutes after infusion, plasma ADM level was also measured. In additional groups, rats were subjected to sepsis by cecal ligation and puncture. At 1.5 hours after cecal ligation and puncture, specific anti–rat ADM antibodies were infused, which completely neutralized the circulating ADM. Various hemodynamic variables were measured 5 hours after cecal ligation and puncture (ie, the early stage of sepsis).

Results: Cardiac output, stroke volume, and microvascular blood flow in the liver, small intestine, kidney, and spleen increased, and total peripheral resistance decreased 0 and 30 minutes after ADM infusion. In addition, plasma levels of ADM increased from the preinfusion level of 92.7 ± 5.3 to 691.1 ± 28.2 pg/mL 30 minutes after ADM infusion, which was similar to ADM levels observed during early sepsis. Moreover, 5 hours after the onset of sepsis, cardiac output, stroke volume, and microvascular blood flow in various organs increased and total peripheral resistance decreased. Administration of anti-ADM antibodies, however, prevented the occurrence of the hyperdynamic response.

Conclusions: The results suggest that increased ADM production and/or release plays a major role in producing hyperdynamic responses during early sepsis. Since our previous studies have shown that vascular responsiveness to ADM decreases in late sepsis, maintenance of ADM vascular responsiveness by pharmacological agents during the course of sepsis may prevent transition from the hyperdynamic to the hypodynamic state.

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Despite advances in the treatment of septic patients, a large number of such patients subsequently die of ensuing septic shock and multiple-organ failure. Septic shock and multiple-organ failure continue to be the most common causes of death in surgical intensive care units, and the incidence of sepsis has increased substantially during the past 2 decades.1,2 Polymicrobial sepsis is characterized by an initial hyperdynamic phase followed by a late hypodynamic phase.2,3 However, the mechanism responsible for producing the transition from the hyperdynamic to the hypodynamic state remains unknown. It is possible that the factor(s) responsible for the transition of hemodynamic responses during sepsis is not fully understood and consequently not prevented, and such factor(s) leads to a progressive deterioration of cell and organ functions and eventual mortality. Thus, it is important to identify the mediator(s) responsible for producing hyperdynamic circulation during the early stage of sepsis.

Adrenomedullin (ADM) is a potent vasodilatory peptide (52–amino acid residues in the human and 50–amino acid residues in the rat) that was first purified in 1993 from human pheochromocytomas.4 The vasodilatory effect of ADM is thought to be primarily mediated through specific ADM receptors,5 which are functionally coupled to adenylate cyclase via G proteins.6 Adrenomedullin is also produced by cultured human smooth muscle cells,7,8 which suggests that ADM may have a role in the regulation of smooth muscle tone.
MATERIALS AND METHODS

The experiments described herein were performed in adherence to the National Institutes of Health guidelines for the use of experimental animals. This project was approved by the Institutional Animal Care and Use Committee of Rhode Island Hospital, Providence.

INTRAVENOUS INFUSION OF SYNTHETIC RAT ADM

Male Sprague-Dawley rats (Charles River Laboratory, Wilmington, Mass), weighing 275 to 325 g, were fasted for 16 hours before the experiment but were allowed water ad libitum. The animals were anesthetized with methoxyflurane inhalation and the left femoral artery was cannulated with polyethylene 50 tubing (Becton Dickinson, Sparks, Md) for measurement of mean arterial pressure and heart rate or withdrawal of blood samples for ADM assays. The right femoral vein was cannulated with polyethylene 10 tubing for ADM administration. Subsequent anesthesia was maintained by intravenous injection of pentobarbital sodium (approximately 30 mg/kg of body weight). After 30 to 60 minutes of stabilization, synthetic rat ADM 1-50 (Peninsula Laboratories, Belmont, Calif) at a dose of 8.5 µg/kg of body weight in 0.5 mL of isotonic sodium chloride solution containing 0.2% bovine serum albumin or 0.5 mL of vehicle (ie, isotonic sodium chloride solution with 0.2% bovine serum albumin) was infused via the femoral venous catheter during 15 minutes at a constant infusion rate (n = 5 per group). Various hemodynamic variables were measured immediately as well as 30 minutes after the completion of ADM or vehicle infusion. The mean arterial pressure and heart rate were monitored by a blood pressure analyzer (Micro-Med, Louisville, Ky). The animals were then killed at the end of the experiment by an overdose of pentobarbital sodium.

ANIMAL MODEL OF EARLY SEPSIS AND ADMINISTRATION OF ANTI-ADM ANTIBODIES

Polymicrobial sepsis was induced in male Sprague-Dawley rats (275-325 g) by CLP according to the method of Chaudry et al. Briefly, rats were fasted overnight before the induction of sepsis but allowed water ad libitum. The animals were anesthetized with methoxyflurane inhalation, and a 2-cm ventral midline incision was made. The cecum was then exposed, ligated just distal to the ileocecal valve to avoid intestinal obstruction, punctured twice, and returned to the abdominal cavity. The abdominal incision was then closed in layers, and the animal received isotonic sodium chloride solution, 3 mL per 100 g of body weight, subcutaneously immediately after CLP (ie, fluid resuscitation). The area of incision was bathed with 1% lidocaine hydrochloride to provide analgesia throughout the study period. Sham-operated-on animals underwent the same surgical procedure except that the cecum was neither ligated nor punctured. Plasma ADM was neutralized by intravenous administration of specific anti-ADM (rat) antisera (ie, polyclonal antibodies; host: rabbit; Peninsula Laboratories) 1.5 hours after CLP. A total of 0.5 mL of isotonic sodium chloride solution containing 8 µL of undiluted anti-ADM antiserum (according to Peninsula Laboratories information, this dose of anti-ADM antibodies is adequate to neutralize plasma ADM during early sepsis) was given and various hemodynamic measures were determined 5 hours after CLP (n = 5 per group). It should be noted that 5 hours after CLP represents a typical point of the early, hyperdynamic stage of polymicrobial sepsis. Vehicle-treated septic animals received 0.5 mL of isotonic sodium chloride solution.

DETERMINATION OF CARDiac OUTPUT AND MICROVASCULAR BLOOD FLOW

Cardiac output (CO) was measured by an indocyanine green dilution technique with a 2.4-F fiberoptic catheter and in vivo hemoreflexometer, as we described previously. The CO was calculated according to the principle of dye dilution. Stroke volume and total peripheral resistance (TPR) were then calculated. Microvascular blood flow on the surface of the liver, small intestine, kidney, and spleen was determined by laser Doppler flowmetry (Laserflo, Model BPM 403A; TSI Inc, St Paul, Minn). The measured microvascular blood flow was the microvascular red blood cell flux in approximately 1 mm² on the surface of each organ with a unit of milliliters per minute per 100 g of tissue, as indicated by the manufacturer. Although this is a reliable technique for determining the alterations in organ surface perfusion, the flow unit suggested by the manufacturer should be considered as arbitrary rather than absolute units.

DETERMINATION OF PLASMA ADM

By means of a radioimmunoassay kit specific for rat ADM (Peninsula Laboratories), plasma levels of ADM were assayed according to the procedure provided by the manufacturer. Briefly, 1.5-mL blood samples were collected into a polypropylene tube containing EDTA (1 mg/mL) and aprotinin (500 kU/mL) at various time points after CLP and plasma was separated immediately. The rat ADM assay does not have any cross-reactivity with human ADM, amylin, or endothelin 1.

STATISTICAL ANALYSIS

One-way analysis of variance and Tukey test or Student t test were used, and the differences were considered significant at P<.05. Results are presented as mean ± SEM.
calligation and puncture (CLP). In addition, up-regulation of ADM production and the onset of the hyperdynamic state occur simultaneously after CLP.3,9 Our hypothesis, therefore, is that ADM plays a major role in producing hyperdynamic circulation during the early stage of polymicrobial sepsis. To test this hypothesis, the present study was carried out to determine whether infusion of synthetic rat ADM in normal animals produces hyperdynamic responses and, if so, whether inhibition of ADM early after the onset of sepsis prevents the occurrence of the hyperdynamic state.

**RESULTS**

The values of mean arterial pressure decreased by an average of 19 mm Hg 15 minutes after the beginning of the synthetic ADM infusion (from an average of 125 to 106 mm Hg). However, this decrease in mean arterial pressure was not statistically different from the preinfusion level (by 1-way analysis of variance). Within 30 minutes after the completion of ADM infusion, mean arterial pressure increased to 125.0 ± 4.4 mm Hg. Similarly, administration of ADM did not produce any significant alterations in heart rate (data not shown).

Administration of ADM at a dose of 8.5 µg/kg in normal animals increased CO by 20% and 41% (P = .02) at 0 and 30 minutes, respectively, after ADM infusion (Figure 1, A). Similarly, stroke volume increased by 21% to 27% (P = .02) at both time points (Figure 1, B). In contrast, TPR values decreased by 22% to 28% (P = .01) 0 and 30 minutes, respectively, after the completion of ADM infusion (Figure 1, C).

**Figure 1.** Alterations in cardiac output (CO) (A), stroke volume (SV) (B), and total peripheral resistance (TPR) (C) before, immediately after (0 minutes), and 30 minutes after adrenomedullin infusion (n = 5). Data are presented as mean ± SEM and compared by 1-way analysis of variance and Tukey test. Asterisk indicates P < .05 vs the values before adrenomedullin infusion.

**Figure 2.** Alterations in microvascular blood flow in the liver (A), small intestine (B), kidney (C), and spleen (D) before, immediately after (0 minutes), and 30 minutes after adrenomedullin infusion (n = 5). Data are presented as mean ± SEM and compared by 1-way analysis of variance and Tukey test. Asterisk indicates P < .05 vs the values before adrenomedullin infusion.
Immediately after ADM infusion, microvascular blood flow in the liver increased significantly (by 56%) and remained elevated 30 minutes after infusion ($P = .01$; Figure 2, A). Similarly, microvascular blood flow increased by 30% to 45% in the small intestine ($P = .004$; Figure 2, B), by 34% to 37% in the kidney ($P = .02$; Figure 2, C), and by 77% to 114% in the spleen ($P = .05$; Figure 2, D) 0 and 30 minutes, respectively, after ADM infusion.

Before the infusion of ADM, plasma ADM level was found to be 92.7 ± 5.3 pg/mL. Thirty minutes after the completion of ADM administration, plasma levels of ADM increased by approximately 6.5-fold to 691.1 ± 28.2 pg/mL ($P < .001$).

Five hours after the onset of sepsis, CO and stroke volume increased by 33% ($P = .02$; Figure 3, A) and 41% ($P = .001$; Figure 3, B), respectively. Administration of anti-ADM antibodies 1.5 hours after CLP, however, prevented the elevation of both measures. There was no difference in CO and stroke volume between animals that received anti-ADM antibodies and sham-operated-on animals (Figure 3, A and B). In addition, TPR decreased by 26% 5 hours after CLP ($P = .02$), and administration of anti-ADM antibodies prevented the reduction in TPR (Figure 3, C).

As illustrated in Figure 4, microvascular blood flow increased by 41% in the liver ($P < .001$), 24% in the kidney ($P = .02$), and 85% in the spleen ($P = .008$) 5 hours after the onset of sepsis. Administration of anti-ADM antibodies 1.5 hours after
Studies have suggested that the potent vasodilatory mediator NO (most likely derived from inducible NO synthase [iNOS]) may be responsible for producing hypotension during septic shock or after administration of endotoxin.12,22 Nitric oxide has also been found to mediate the redistribution of intrarenal blood flow during bacteremia.18 However, it remains unclear whether this molecule and/or other vasodilatory agents are responsible for producing the hyperdynamic state observed during the early stage of polymicrobial sepsis. In this regard, our recent studies have indicated that the release of endothelium-derived NO decreased during both early and late stages of sepsis.15,16 In addition, it has been demonstrated that plasma levels of nitrate or nitrite (stable products of NO, reflecting iNOS activity) increased several hours after the onset of hyperdynamic circulation in the CLP model of sepsis.3,17 In view of these findings, it is unlikely that the alteration in NO production is responsible for initiating the hyperdynamic circulation during the early stage of sepsis. Nor is it likely that NO is responsible for producing the transition from hyperdynamic circulation to hypodynamic circulation during sepsis. Although circulating levels of prostacyclin increase significantly during early sepsis, administration of the cyclooxygenase inhibitor indomethacin or the specific prostaglandin synthase inhibitor tranilcipromine does not prevent the occurrence of hyperdynamic circulation under such conditions.18 Thus, it is likely that mediator(s) other than NO and prostacyclin are responsible for producing hyperdynamic responses during early sepsis. The present study, therefore, was undertaken to determine whether the novel vasodilatory peptide ADM plays any significant role in producing hyperdynamic circulation during the early stage of sepsis.

The results indicate that administration of synthetic rat ADM at a dose similar to that observed during early sepsis,7 which did not produce significant alterations in mean arterial pressure or heart rate, markedly increased CO and stroke volume while decreasing TPR. In addition, infusion of ADM increased microvascular blood flow in the liver, small intestine, kidney, and spleen. Furthermore, CO, stroke volume, and microvascular blood flow in various organs increased while TPR decreased significantly 5 hours after the onset of sepsis. Administration of specific anti-ADM antibodies 1.5 hours after CLP, however, prevented the occurrence of the hyperdynamic response under such conditions. These findings, therefore, suggest that increased ADM production or release plays a major role in producing hyperdynamic circulation during the early stage of sepsis. It should be noted that anti-ADM antibodies were administered 1.5 hours after the onset of sepsis. This time point was selected because our recent studies have shown that plasma levels of ADM increased 2 hours but not 1.5 hours after CLP.9 Anti-ADM antibodies were administered just before the increase in circulating ADM to neutralize ADM activity. However, it remains unknown whether administration of anti-ADM antibodies at a later time point, such as 5 hours after CLP, reverses hyperdynamic and hypocardiovascular responses.

Studies have indicated that, in human subjects, ADM production and/or release increases after septic shock7,8 as well as other pathophysiological conditions, such as ischemic injury and organ failure.19,20 In addition, the increase in ADM gene expression and its production can be observed after administration of bacterial endotoxin21 and proinflammatory cytokines, such as tumor necrosis factor and interleukin 1.22 Using the CLP model of sepsis, we recently demonstrated that plasma levels of ADM increased as early as 2 hours after CLP and were sustained even 30 hours after the onset of sepsis.9 Moreover, the ADM levels in the heart and aorta were found to be elevated during sepsis.23 Studies by other investigators have shown that ADM gene expression is up-regulated in the small intestine, liver, lungs, aorta, heart, and skeletal muscle after endotoxin administration.21,24 In this regard, we have shown that ADM gene expression increased in the small intestine, left ventricle, and thoracic aorta, but not in the liver and kidney, during the early stage of sepsis.9 Although endotoxin plays a role in producing various cardiovascular responses during sepsis, the observed plasma levels of ADM under such conditions are much lower than those encountered after endotoxemia or endotoxic shock.25 Thus, it appears unlikely that endotoxin is the sole factor responsible for ADM production during sepsis. Since endotoxin and proinflammatory cytokines synergistically stimulate the production of ADM,22 and since circulating levels of tumor necrosis factor, interleukin 1, and interleukin 6 increase significantly during sepsis26-28 or after endotoxin administration,29 it appears that the increased circulating levels of proinflammatory cytokines and endotoxin are responsible for the up-regulated ADM production during sepsis.

Parkes30 reported that administration of ADM in conscious sheep increased CO and coronary blood flow and decreased peripheral vascular resistance. Moreover, administration of ADM in rats increased CO and blood flow in the heart, lungs, kidney, and small intestine while decreasing vascular resistance.31 However, an increase in the dosage of ADM and/or its infusion rate may induce systemic hypotension.30,31 In the present study, a relatively small dose of ADM was administered to normal rats, in contrast to the study by He et al,31 in which ADM infusion produced hypotension. It should be noted that the effects of ADM on the cardiovascular system were determined only 0 and 30 minutes after the completion of ADM infusion. Thus, it remains unknown how long ADM-induced hypocardiovascular responses persist after ADM administration and whether an elevated plasma level of ADM is required to maintain the hyperdynamic circulation.

Although we have not determined ventricular performance after ADM infusion in normal animals or in septic animals after administration of anti-ADM antibodies in the present study, Zhou et al32 recently reported that ventricular contractility (as indicated by the maximum rate of left ventricular pressure increase and decrease) increased significantly during the early stage of sepsis. In addition to its effects on the vascula-
Despite major advances in the treatment of trauma victims, a large number of those patients subsequently die of sepsis, septic shock, and the ensuing multiple-organ failure. It is encouraging, however, that the complex pathobiology of sepsis is becoming better understood as more investigations are being reported. Through such studies, information may be forthcoming that will lead to better treatment of septic patients. Polymicrobial sepsis is characterized by an early, hyperdynamic phase followed by a late, hypodynamic phase. It is, therefore, important to determine the mechanism responsible for producing the hyperdynamic response during early sepsis and the factors contributing to the transition from the hyperdynamic to the hypodynamic stage of sepsis. In this regard, the present study indicates that the increased ADM production or release plays a major role in producing hyperdynamic circulation during the early stage of sepsis. In addition, recent studies have demonstrated that the vascular responsiveness to ADM decrease significantly during the late, hypodynamic phase of sepsis, whereas ADM-induced vascular relaxation remains unaltered during the early, hyperdynamic phase. It is therefore possible that a reduced vascular responsiveness to ADM (most likely via down-regulation of ADM receptors) is responsible for producing the transition from the hyperdynamic to the hypodynamic stage of sepsis. In view of this, we propose that maintenance of the vascular responsiveness to ADM by the administration of pharmacological agents during the course of sepsis may prevent the transition from the hyperdynamic to the hypodynamic state of sepsis, thereby maintaining hemodynamic stability during sepsis and preventing the onset of septic shock and multiple-organ failure.

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Reprints: Ping Wang, MD, Center for Surgical Research, Rhode Island Hospital, Middle House II, 593 Eddy St, Providence, RI 02903 (e-mail: pwang@lifespan.org).

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DISCUSSION

Michael A. West, PhD, MD, Minneapolis, Minn: What the authors have shown us is that possibly this, at least for many of us, new molecule, the ADM, may in fact have an important hemodynamic role. These data presented here are very tantalizing, but there are a number of important controls that I think are missing, and certainly I’m sure that the laboratory is going to be pursuing further studies in this.

Some of the specific questions that I would like to ask relate to the fact that you saw, at least in your infusion of ADM, some very significant effects, even at time zero, and I want to kind of concentrate on that. I am wondering what the mechanism for this is.

You showed a decrease in peripheral resistance, an increase in stroke volume, and a borderline increase, as far as statistical significance, in terms of cardiac output, even at time zero, and I am wondering how that ADM response might be mediated. In your manuscript the authors talk about the possibility that some of the mechanisms for ADM may involve increases in cyclic AMP within the cells and may in fact involve increase in calcium in the cells and activation of a constitutive nitric oxide synthase.

My next question is, if nitric oxide synthase is involved, have you looked at whether there are any associated increased levels of nitrate or nitrite in those cells?

You showed us the ADM levels at 30 minutes after the infusion. You didn’t show us any ADM levels at time zero. And the other control that I would like to see, and perhaps you have some data on it, is in terms of your ADM infusion. Did you try administering your antibody to show the same sort of specificity that is implied in your CLP model? Because you could block your CLP effects with the antibody, could you prevent at time zero and 30 minutes your ADM infusion effects with the antibody, or is there something else going on?

Finally, in terms of the time course that we saw, again, with the infusion, you showed us 30 minutes. Do you have any intermediate time points to suggest how quickly this response comes in? It is a very interesting hypothesis and interesting data.

Dr Wang: Dr West, in terms of the control group, you are right, we have a long way to look at the effect of ADM in terms of its work during sepsis. The mechanism of ADM, as you pointed out, is through an increase of cyclic AMP and an increase in nitric oxide release, and we have not measured nitrate and nitrite in this particular study; however, previously we have shown that nitrate and nitrite levels in the circulation do not increase at 2 and 5 hours; however, they increased significantly at 10 and 20 hours. Since hyperdynamic circulation occurs as early as 2 hours, the increased nitrate and nitrite at 10 hours do not affect the time course. In other words, the increased iNOS-induced, iNOS-derived nitric oxide may not be the reason for the occurrence of hyperdynamic circulation.

You also asked the time course of ADM, whether or not at a zero time the cardiac output increased; however, that increase was not a significant difference.

Another question you mentioned is the administration of antibody in the ADM-infused animal, whether or not that administration of ADM antibody can prevent the occurrence of hyperdynamic circulation. We have not conducted such studies; however, the sepsis studies indicate that when you give antibody at the early stage of sepsis, you prevent the hyperdynamic response. Since ADM is responsible for the increase in cardiac output, stroke volume, and blood flow, we would expect that if you give antibody at the time of perfused ADM, you may see no increase in cardiac output and blood flow. In other words, the antibody in that setting may prevent the occurrence of hyperdynamic response.

The time course we did is zero time and 30 minutes. We did not look at the median time points, for instance, 15 minutes. I would expect that at 15 minutes after ADM infusion, the cardiac output and the blood flow probably increased as we showed at 30 minutes.

Declan Carey, MD, Cardiff, Wales: Dr Wang, you stated in one of your method slides that your anti–rat ADM antibody completely blocked the ADM response and then very clearly measured in your cecal ligation and puncture model the levels of ADM; however, you didn’t provide us proof in the treated animals that you were able to block the ADM levels by measuring the levels in the treated animals. Why did you not do that?

Dr Wang: The reason we did not measure ADM levels in ADM antibody–treated animals is because the measurement was radioimmunoassay. One way to look at it is to look at the physiological response of the blood sample from dead animals. One of the experiments we should do is take a blood sample from the ADM antibody–treated septic animals in order to stimulate blood vessels to see whether or not we will see a vascular relaxation.

Richard J. Howard, MD, Gainesville, Fla: Let me ask a question that seems too obvious: Is that why it is probably not a good question? Does ADM, as the name suggests, adrenomedullin, come from the adrenal gland or does it come from other sites? If it comes only from the adrenal gland, the obvious experiment is what happens to adrenalectomized animals, and have you studied those?

Dr Wang: You are right; initially it was isolated from the tumor cell line from the adrenal gland; however, we have done several studies to look at the tissue levels of adrenal level protein as well as the gene expression. It basically expressed in most organs and initially in other organs except macrophage. However, recently, even in this cell population, we still can see adrenomedullin.

R. Neal Garrison, MD, Louisville, Ky: I am curious as to how you define or how you measure peripheral vascular resistance. The main peripheral vascular resistant bed is skeletal muscle, which accounts for about 90% of vascular resistance beds; the liver and the kidney and the splanchnic organs are organ-specific perfusion beds. Did you measure the effect of this compound on perfusion to skeletal muscle beds?

Dr Wang: How did we measure total peripheral resistance? We used a classic physiological measure: peripheral resistance is equal to mean cardiac output divided by blood pressure minus central venous pressure.

In terms of your second question, we have not specifically measured organ resistance. That can be calculated by the blood flow to that organ as well as the blood pressure to that specific organ.