

Cardiac and Renal Effects of Levosimendan, Arginine Vasopressin, and Norepinephrine in Lipopolysaccharide-treated Rabbits

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Background: Because sepsis-induced myocardial dysfunction related to sepsis is at least partially related to a decrease in cardiac myofilament response to calcium, the use of the new myofilament-calcium sensitizer, levosimendan, has been proposed. In addition, arginine vasopressin is increasingly proposed as a vasopressor in septic patients, although data on its effects on cardiac function are still scarce. The aim of the current study was to assess, invasively and noninvasively, whether levosimendan, arginine vasopressin, and norepinephrine, either alone or combined, may modify sepsis-induced myocardial dysfunction and renal hemodynamics.

Methods: Thirty-six hours after lipopolysaccharide or saline administration, rabbits were studied either after slight sedation for echocardiography or after general anesthesia with sodium pentobarbital for the following measurements: aortic flow velocity and maximum acceleration of blood flow in the ascending aorta and renal macrocirculation and microcirculation.

Results: Levosimendan improved, within 30 min of administration, both maximum acceleration of blood flow by $20 \pm 12\%$ ($n = 8$; $P < 0.05$) and left ventricular shortening fraction by a similar extent. Furthermore, low doses of arginine vasopressin markedly deteriorated cardiac function *via* an afterload-independent mechanism, even when animals were pretreated with levosimendan, whereas norepinephrine showed no detrimental effects on cardiac function. The study also showed that norepinephrine often improved renal medullary blood flow, whereas arginine vasopressin consistently decreased it.

Conclusion: Levosimendan and norepinephrine both exert beneficial effects in endotoxemic animals and should be further explored in human sepsis trials.

ALTERATIONS in cardiac function and peripheral vascular tone are consistently present in severe sepsis and in septic shock¹⁻⁵ with or without fluid resuscitation.^{6,7} A recent Consensus conference emphasized that early treatment of septic shock includes adequate fluid loading associated or not with pressors and/or cardiac ino-

tropes.⁴ Among vasopressors, dopamine or more frequently norepinephrine is the treatment of choice.^{8,9} In North America, arginine vasopressin is emerging as an alternative therapy of vasodilatory shock due to systemic inflammatory response syndrome.¹⁰⁻¹² Although norepinephrine and arginine vasopressin seem to be the two most efficient vasopressors, doubts remain about their potential detrimental effects on septic cardiac dysfunction, especially *via* the increase in left ventricular afterload.

In studies focused on potential mechanisms that may contribute to sepsis-induced cardiomyopathy, it was recently hypothesized that, during sepsis, cardiac myofilaments phosphorylation decreases myofibrillar-calcium sensitivity.^{1,13} The latter may contribute to the depression of cardiac contractility probably *via* a modulation of the regulatory action of troponin I on troponin C.¹ It was therefore proposed that an agent that may improve cardiac myofilament response to calcium (*i.e.*, a calcium-sensitizing agent) may specifically improve contractility in sepsis. Levosimendan is a calcium-sensitizing agent that is indicated for use in patients with acutely decompensated heart failure.^{14,15} The calcium-sensitizing effects of levosimendan are mediated through its calcium-dependent binding to cardiac troponin C,^{16,17} which produces an increase in the contractile force of the cardiac myocytes¹⁸ without increases in intracellular calcium concentrations¹⁹ and with little or no increase in myocardial oxygen consumption.²⁰ In a recent study, pretreatment with levosimendan in pigs subjected to endotoxin shock improved cardiac output and systemic and gut oxygen delivery.²¹ Other authors showed that levosimendan may attenuate, *ex vivo*, myocardial dysfunction in hearts isolated from guinea pigs, 4 h after lipopolysaccharide administration.²² In both previous studies, however, levosimendan was tested on animal models before or few hours after endotoxin administration. Therefore, our aim was to establish the role of levosimendan in treating sepsis-related myocardial failure by studying its effects on a chronic animal model of endotoxemia that was previously shown to mimic human septic cardiomyopathy.^{3,13}

Accordingly, our aim was to assess the cardiovascular effects of levosimendan alone or of arginine vasopressin or norepinephrine either alone or, most importantly, combined with levosimendan in lipopolysaccharide-treated rabbits with a cardiovascular depression. Our study showed that (1) levosimendan improved cardiac

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systolic function in lipopolysaccharide-treated rabbits, and (2) arginine vasopressin, although at doses that only slightly increased mean arterial pressure, markedly deteriorated cardiac function even when combined with levosimendan, whereas norepinephrine showed no detrimental effects on cardiac function.

Materials and Methods

Reactives and Drugs

The endotoxin mixture was prepared with *Escherichia coli*, *Salmonella enteritidis*, and *Salmonella minnesota* lipopolysaccharides (Sigma Chemical Co., St. Louis, MO) as previously described.^{3,13} The day of experiment, levosimendan powder was solubilized with 0.05 M NaHCO₃ and then diluted in 0.9% saline to obtain a final solution of 200 µg/ml. A 1-mg/ml stock solution of arginine vasopressin (Sigma Chemical Co., St. Louis, MO) was prepared and stored at -80°C until use. Norepinephrine (Noradrénaline Aguettant®; Laboratoire Aguettant; Fougères, France) was prepared from a stock solution of 2 mg/ml.

Animals

Care of the animals conformed to the recommendations of the Helsinki Declaration and the study was performed in accordance with the regulations of the official edict of the French Ministry of Agriculture. Fifty-seven male White New Zealand rabbits (Charles River, L'Arbresle, France) weighing 2.2 ± 0.1 kg were housed in individual cages in a controlled environment with free access to food and water for at least 4 days before experimentation. Thirty-six hours before starting treatment and hemodynamic measurements, 600 µg/kg lipopolysaccharide mixture or saline (control) was injected *via* a 22-gauge catheter placed in a marginal ear vein, as previously described.^{3,13} Animals were fasted the night before experimentation.

Overall mortality was 19% (n = 9) in lipopolysaccharide group, quasi-exclusively during the 36 h after lipopolysaccharide administration, and 0% in control. Therefore, data presented here are the summary of the 38 animals that survived in lipopolysaccharide group and 10 control animals.

Animal Preparation

In the main series of rabbits, general anesthesia was induced by intravenous injection of sodium pentobarbital (CEVA Santé Animale, Libourne, France). The dose of sodium pentobarbital required to achieve an optimal anesthesia without deleterious hemodynamic effects was 21 ± 6 mg/kg in the lipopolysaccharide group and 35 ± 6 mg/kg in the control group ($P < 0.0001$) for the induction of anesthesia and 6 ± 2 mg · kg⁻¹ · h⁻¹ in the lipopolysaccharide group and 13 ± 1 mg · kg⁻¹ · h⁻¹ in control group ($P < 0.0001$) to maintain anesthesia. After

tracheostomy, animals were mechanically ventilated with 100% oxygen (Rodent ventilator; Harvard Apparatus, Boston, MA). Rectal temperature was maintained near 38.5°C by using a warming pad. Catheters were inserted in right carotid and right jugular vein, and a 20-MHz pulsed Doppler flow velocity probe (4 mm internal diameter) was positioned around the ascending aorta.

A surface laser Doppler probe (Transonic System, Ithaca, NY) was placed on the renal convexity between the capsule and the cortex. A 26-gauge laser Doppler needle probe (Transonic Needle System) was inserted in the medulla after making a small hole in the capsule of at least 1 cm far away from the cortical probe. The two laser Doppler probes were connected to a Doppler flow meter (BLF21D; Transonic System). The mean signals from renal artery, cortex, and medulla were measured continuously.

In another series of spontaneously breathing rabbits, transthoracic echocardiography (Vivid7 General Electric; Horten, Norway) with a 12-MHz transducer was performed during light sedation with sodium pentobarbital.

Hemodynamic Variables and Calculations

In the series of rabbits under general anesthesia, the following variables were measured. Heart rate (beats/min), arterial blood pressure (mmHg), systolic aortic blood flow velocity (sAoV; cm/s), systolic and diastolic renal artery blood flows (ml/min), and cortical and medullary blood flows (in arbitrary units, Tissue Perfusion Units) were continuously measured at a sampling rate of 1,000 s⁻¹, and recorded on a computer (Macintosh Power PC6745) with an analogic/digital transducer (Biopac Systems MP100, Goleta, CA). Baseline aortic blood flow zero velocity was verified during the diastolic time. Aortic acceleration was calculated as the sAoV first derivative. Peak values of sAoV and aortic acceleration (maximal aortic acceleration [Gmax]) were used as indexes of systolic function.²³⁻²⁵

In the series of spontaneously breathing rabbits, transthoracic echocardiography was applied in parasternal long-axis view at a frequency of 120 Hz. It allowed the following measurements in M mode: (1) left ventricular end-diastolic diameter, defined as the largest left ventricle diameter; (2) left ventricular end-systolic diameter, defined as the smallest left ventricle diameter; and (3) left ventricle fraction shortening (%), as

$$\frac{\text{left ventricular end diastolic diameter} - \text{left ventricular end systolic diameter}}{\text{left ventricular end diastolic diameter}} \times 100\%.$$

The echocardiographer was blinded to treatment.

Study Design

In the rabbits under general anesthesia, after a recovery period of 30–45 min after surgery, three consecutive

Table 1. Baseline Values 36 h after Lipopolysaccharide or Saline Injection

	Lipopolysaccharide-treated Rabbits	Saline Rabbits	P Value
Weight changes vs. baseline, g	-239 ± 65	-45 ± 95	< 0.001
Heart rate, beats/min	254 ± 26	322 ± 16	< 0.001
mBP, mmHg	78 ± 25	89 ± 3	NS
sAoV, m/s	0.84 ± 0.18	0.91 ± 0.14	NS
Gmax, m/s ²	50 ± 16	64 ± 11	< 0.05
sRenal BF, ml/min	27 ± 10	29 ± 4	NS
dRenal BF, ml/min	12 ± 7	15 ± 3	NS
Plasma lactate, mM	2.9 ± 2.2	2.0 ± 0.7	< 0.05
Arterial pH, U	7.29 ± 0.1	7.38 ± 0.06	< 0.05
Paco ₂ , mmHg	50 ± 19	42 ± 6	NS
Pao ₂ , mmHg	281 ± 117	376 ± 88	NS
Plasma AVP, pg/ml	13.1 ± 11.4	55.2 ± 43.2	0.003

Values were collected from lipopolysaccharide-treated rabbits (n = 34, except for arginine vasopressin [AVP; n = 12]) and saline rabbits (n = 6). P values were calculated by Mann-Whitney test.

dRenal BF = diastolic renal blood flow; Gmax = maximal aortic acceleration; mBP = mean arterial blood pressure; NS = not significant; Paco₂ = arterial carbon dioxide tension; Pao₂ = arterial oxygen tension; sAoV = systolic aortic blood flow velocity; sRenal BF = systolic renal blood flow.

hemodynamic measurements were performed over 30 min to assess the stability of the preparation. For each variable, the average value between the three recordings was considered as baseline. Twenty milliliters saline was infused in 5 min to assess cardiac response to fluid challenge. Hemodynamic measurements were repeated at the end of fluid challenge.

In a first set of experiments, rabbits in the lipopolysaccharide group (n = 8) and control rabbits (n = 6) received a continuous infusion of levosimendan at a dose previously described in animals (200 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$)²¹ during 4 h. In these animals, levosimendan plasma concentration, measured as previously described,²⁶ was 286 ± 92 ng/ml at 60 min and slightly further increased to 336 ± 84 ng/ml at 240 min.

In a second set of experiments, the effects of pressor agents were tested on cardiac performance in lipopolysaccharide group. Thus, only moderate doses of norepinephrine and arginine vasopressin, with a moderate impact on blood pressure to limit the effect of afterload modification, were tested. Lipopolysaccharide-treated animals (n = 26) were randomized and separated in four groups, receiving, respectively, levosimendan (200 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) alone during 30 min followed by the combination of levosimendan and norepinephrine (1 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, usual dose in human studies) for an additional 90 min (n = 7); saline solution during 30 min followed by norepinephrine (1 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) alone during 90 min (n = 6); levosimendan (200 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) alone during 30 min followed by the combination of levosimendan with arginine vasopressin (0.005 U · $\text{kg}^{-1} \cdot \text{min}^{-1}$, determined in pilot studies to induce similar changes in blood pressure than norepinephrine [1 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$] in lipopolysaccharide-treated rabbits) for an additional 90 min (n = 6); or saline solution during 30 min followed by arginine vasopressin (0.005 U · $\text{kg}^{-1} \cdot \text{min}^{-1}$) alone during 90 min (n = 7).

Hemodynamic measurements were repeated every 15

min during treatment. Blood samples were drawn from the carotid artery during the baseline period (before fluid challenge) and at the end of the study period for biologic assessment. The following measurements were performed in plasma: glucose, lactate (spectrophotometric technique, Ektachem 950 IRC system; Johnson & Johnson, Strasbourg, France); blood gas (ABL-30; Radiometer, Copenhagen, Denmark); arginine vasopressin²⁷ and troponin I (by immunoenzymatic technique as previously described in rabbits²⁸).

Spontaneously breathing rabbits included four lipopolysaccharide-treated animals and four control animals. Echocardiographic measurements were performed before and 30 min after levosimendan administration (200 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$).

Statistical Analysis

Results were expressed as mean ± SD. Intergroup and intragroup differences were tested, in data presented in all figures, by two-way and one-way analyses of variance for repeated measures, respectively. A Mann-Whitney test was performed when intergroup differences were tested at one time point, as in table 1. A P value less than 0.05 was considered significant.

Results

Myocardial Effects of Lipopolysaccharide Treatment

In vivo injection of lipopolysaccharide altered body weight and metabolic parameters, 36 h after treatment, compared with saline rabbits, as summarized in table 1.

Figure 1 shows that cardiac systolic function was impaired 36 h after lipopolysaccharide treatment as previously reported.^{3,13} Fluid challenge-induced increase in sAoV was smaller in the lipopolysaccharide group than in the control group (P < 0.05). In addition, the index of cardiac systolic function Gmax was lower (table 1) and remained so after fluid challenge in the lipopolysaccha-

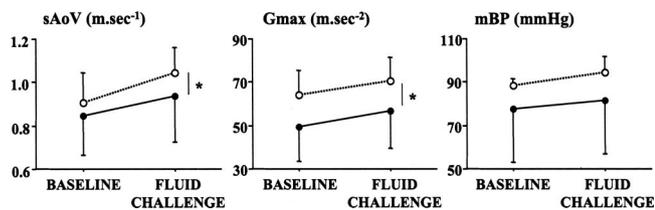


Fig. 1. Effect of fluid challenge on systemic hemodynamics. Measurements were performed before (baseline) and at the end of 20 ml intravenous saline administered in 5 min (fluid challenge), in lipopolysaccharide ($n = 34$; ●) and control ($n = 6$; ○) rabbits. Data are presented as mean \pm SD. * $P < 0.05$ interaction between groups by two-way analysis of variance for repeated measures. Gmax = maximal acceleration of aortic blood flow; mBP = mean arterial blood pressure; sAoV = systolic aortic blood flow velocity.

ride group compared with the control group (fig. 1; $P < 0.05$).

Lipopolysaccharide-induced impairment in myocardial function was confirmed by echocardiography in spontaneously breathing rabbits: Left ventricular shortening fraction was impaired in lipopolysaccharide-treated ($n = 4$) compared with control ($n = 4$) animals (28 ± 2 vs. $36 \pm 1\%$, respectively; $P < 0.05$, Mann-Whitney test).

Figure 1 also shows that mean arterial pressure was lower, although not significant, in the lipopolysaccharide group than in the control group and remained lower after fluid challenge.

Beneficial Effects of Levosimendan on Cardiac Performance in Lipopolysaccharide-treated Rabbits

As shown in figure 2, the calcium-sensitizer levosimendan improved myocardial contractile function in both lipopolysaccharide-treated and control animals. In lipopolysaccharide-treated animals, levosimendan improved both sAoV and Gmax by $+13 \pm 8\%$ and $20 \pm 12\%$, respectively ($P < 0.05$ for both), as early as 30 min after the start of treatment. Improvement in contractile function persisted during the whole study period. During the same period, levosimendan induced a decrease in mean arterial pressure in all studied animals (fig. 2) and an increase in heart rate: $+33 \pm 13$ beats/min, corresponding to $6 \pm 10\%$ in lipopolysaccharide-treated animals, and $+19 \pm 7$ beats/min, corresponding to $6 \pm 2\%$ in control animals ($P < 0.05$ for both).

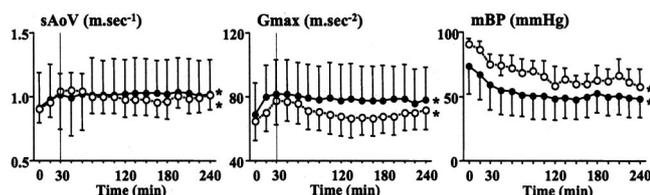


Fig. 2. Myocardial and vascular effects of levosimendan in lipopolysaccharide ($n = 8$; ●) and control ($n = 6$; ○) rabbits. For all six curves, * $P < 0.05$ by one-way analysis of variance for repeated measures. Gmax = maximal acceleration of aortic blood flow; mBP = mean arterial blood pressure; sAoV = systolic aortic blood flow velocity.

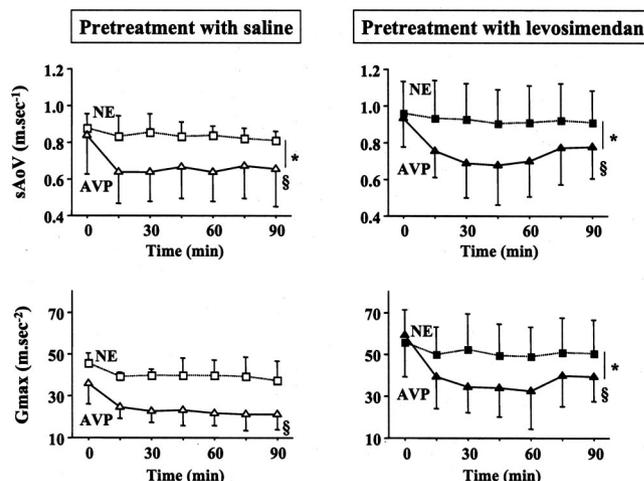


Fig. 3. Effect of norepinephrine (NE) or arginine vasopressin (AVP) on cardiac function after 30 min pretreatment with saline or levosimendan in lipopolysaccharide-treated rabbits. Measurements were performed every 15 min during a 90-min infusion. As described in the Results section, NE and AVP increased mean arterial pressure by 7 mmHg on average, within the first 15 min, regardless of the pretreatment. NE alone (□; $n = 5$), levosimendan + NE (■; $n = 7$), AVP alone (Δ; $n = 7$), levosimendan + AVP (▲; $n = 5$). * $P \leq 0.05$ interaction between the two groups, two-way analysis of variance for repeated measures. § $P < 0.05$, one-way analysis of variance for repeated measures. Gmax = maximal acceleration of aortic blood flow; sAoV = systolic aortic blood flow velocity.

A beneficial effect of levosimendan on cardiac performance was confirmed by echocardiography, showing an improvement in left ventricle shortening fraction in lipopolysaccharide-treated and control rabbits (combined data, $n = 8$, from 32 ± 4 to $36 \pm 2\%$; $P < 0.05$).

Hemodynamic Effect of Norepinephrine, Arginine Vasopressin, and Levosimendan in Lipopolysaccharide-treated Rabbits

Mean arterial blood pressure increased significantly with norepinephrine by $+7 \pm 9$ mmHg ($n = 13$; $P = 0.01$) but not with arginine vasopressin by $+7 \pm 12$ mmHg ($n = 13$; $P =$ not significant). However, diastolic arterial blood pressure improved with both norepinephrine ($+8 \pm 10$ mmHg) and arginine vasopressin ($+12 \pm 12$ mmHg; $P < 0.05$ for each drug).

Figure 3 shows cardiac effects of norepinephrine or arginine vasopressin associated or not with levosimendan in lipopolysaccharide-treated animals. Although norepinephrine never altered myocardial function indexes over 90 min of treatment, arginine vasopressin induced a striking deterioration in both sAoV and Gmax in all arginine vasopressin-treated rabbits even when pretreated with the inotropic agent levosimendan (all $P < 0.05$). In addition, norepinephrine did not alter heart rate when associated or not with levosimendan, whereas arginine vasopressin did decrease heart rate by -21 ± 22 beats/min over 90 min in all arginine vasopressin-treated animals ($P < 0.05$). Furthermore, ejection time remained unchanged over the treatment period in all

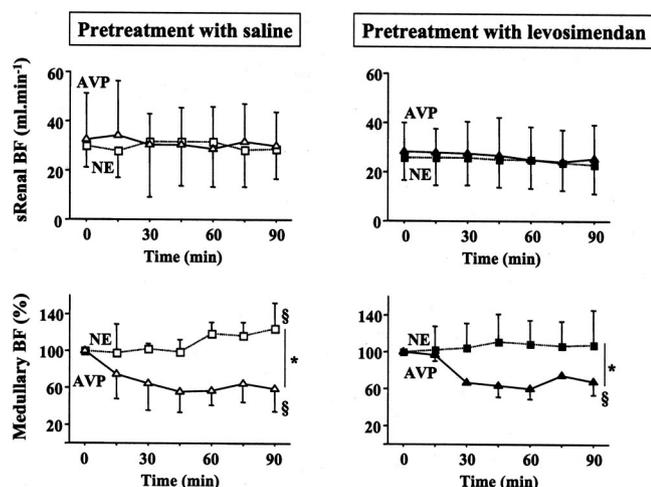


Fig. 4. Effect of norepinephrine (NE) or vasopressin (AVP) on renal perfusion after 30 min pretreatment with saline or levosimendan in lipopolysaccharide-treated rabbits. Same *n* as figure 3. * $P < 0.01$, interaction between the two groups, two-way analysis of variance for repeated measures. § $P < 0.05$, one-way analysis of variance for repeated measures. Medullary BF = medullary renal blood flow, expressed as percentage changes compared with baseline (before NE or AVP administration); sRenal BF = systolic renal arterial blood flow (of note, neither NE nor AVP altered diastolic renal blood flow (data not shown).

norepinephrine-treated animals (137 ± 38 ms before and 129 ± 37 ms after 90 min of treatment) and in all arginine vasopressin-treated animals (119 ± 32 ms before and 124 ± 36 ms after 90 min of treatment).

Renal Effects of the Vasoactive Drugs

As shown in table 1, no significant difference was observed in renal blood flow in basal conditions between lipopolysaccharide-treated and control rabbits. Levosimendan alone did not alter systolic or diastolic renal blood flow or cortical or medullary perfusion within 30 min of administration in lipopolysaccharide-treated rabbits (data not shown). Arginine vasopressin and norepinephrine also did not alter systolic (fig. 4) or diastolic (data not shown) renal blood flow, regardless of the use of levosimendan in lipopolysaccharide-treated animals. However, as shown in figure 4, norepinephrine did increase medullary tissue perfusion in the absence of levosimendan ($P < 0.05$) but not when combined with levosimendan, whereas arginine vasopressin did consistently decrease medullary perfusion whether it was used with or without levosimendan ($P < 0.05$). Neither arginine vasopressin nor norepinephrine altered cortical perfusion (data not shown).

Metabolic Measurements

As shown in table 1, plasma arginine vasopressin concentration was lower in lipopolysaccharide-treated rabbits compared with control rabbits.

Interestingly, differences were observed in the time course of glycemia and lactatemia among the four groups of lipopolysaccharide-treated animals ($n = 26$).

Although glycemia was similar among the four groups at baseline (9.4 ± 2.4 mM), norepinephrine-treated animals had a higher glycemia (13.3 ± 3.8 mM) compared with arginine vasopressin-treated rabbits (9.1 ± 2.2 mM; $P < 0.01$) after 90 min of treatment. By contrast, although lactate was similar among the four groups at baseline (2.9 ± 2.2 mM), norepinephrine-treated animals had a lower lactate concentration (3.3 ± 3.0 mM) compared with arginine vasopressin-treated rabbits (4.8 ± 2.9 mM; $P < 0.05$) after 90 min of treatment. Troponin I did not differ significantly among the four groups at any time (data not shown).

Discussion

Our study showed that (1) levosimendan improved cardiac function in endotoxemic rabbits; (2) low doses of arginine vasopressin markedly deteriorated cardiac function, even when animals were pretreated with levosimendan, whereas norepinephrine showed no detrimental effects on cardiac function; and (3) norepinephrine and arginine vasopressin had opposite effects on renal medullary blood flow.

Levosimendan is a myofilament-calcium sensitizer that improves cardiac contractility with coronary and peripheral vasodilatation.²⁹ The primary mechanism of action for levosimendan is through Ca^{2+} sensitization of contractile proteins, although it does not affect the intracellular Ca^{2+} concentrations.^{18,19} Our group showed that the profound myocardial depression observed in sepsis was mostly related to intrinsic alterations in cardiac myocytes function rather than direct cardiodepressing effects of circulating agents.^{2,3,13} Our group and others showed that intrinsic alteration of cardiac myocyte function in sepsis was not related to changes in intracellular calcium concentration but rather to a decrease in myofilaments' response to calcium in several animal species.^{1,2,13} We further showed that the latter was related to an increased phosphorylation of troponin I^{2,13} that modulated the regulatory action of troponin I on troponin C. In the current study, we showed both by invasive measurements in anesthetized animals and by echocardiography in spontaneously breathing animals that levosimendan markedly improved sepsis-induced myocardial dysfunction. However, as shown in figure 2, levosimendan decreased mean arterial pressure in our endotoxemic rabbits over the 4-h study period. Although one may suggest that levosimendan-induced improvement in indexes of cardiac performance could be related to decrease in left ventricle afterload, figure 2 shows that the increase in maximum acceleration of aortic blood flow in the ascending aorta was maximum at 15 min at the time when mean arterial pressure was only slightly decreased in endotoxemic animals. In addition, although mean arterial pressure further decreased after 30 min,

neither aortic velocity nor maximum acceleration changed. Accordingly, our data showed that levosimendan directly improved cardiac performance in our endotoxemic animal model, regardless of the level of arterial pressure. Further experiments should be performed in septic patients to confirm the beneficial effects of levosimendan on sepsis-induced myocardial depression. Care should be taken to evaluate and maintain, if needed, organ perfusion pressure with vasoactive agent, optimally norepinephrine, as shown below.

Arginine vasopressin is increasingly used to restore arterial blood pressure in human septic shock, and promising data recently were recently obtained in animal studies of hemorrhagic shock and cardiopulmonary arrest.^{12,30} Relative vasopressin deficiency was repeatedly found in patients with severe sepsis.^{31,32} A similar result was found in our animal model, with baseline plasma arginine vasopressin concentration lower in lipopolysaccharide-treated than in control animals (table 1). Few human studies dealt with the use of arginine vasopressin in septic shock, and data on myocardial function are still missing. Two randomized studies have shown that 0.01–0.04 U/min arginine vasopressin was effective in the correction of sepsis-induced hypotension.^{10,33} Although cardiac output remained unchanged in the two studies, this does not exclude an intrinsic deterioration of myocardial contraction. To assess arginine vasopressin effects on myocardial contraction in sepsis, we performed beat-by-beat measurements of systolic aortic velocity and maximal aortic acceleration, previously described as good indexes of myocardial contraction.^{23–25} Our study shows that despite a slight and nonsignificant increase in mean arterial pressure (+7 mmHg), arginine vasopressin alone induced a marked decrease in heart rate as previously described³⁴ but more importantly deteriorated the indexes of myocardial contraction: sAoV and Gmax. Because ejection time remained constant during arginine vasopressin infusion, the decrease in sAoV also represented a deterioration in stroke volume. Therefore, altogether, the decrease in both heart rate and stroke volume associated with the administration of arginine vasopressin imply a marked deterioration in cardiac output in our lipopolysaccharide-treated rabbits. Interestingly, pretreatment with the positive inotropic agent levosimendan could not prevent arginine vasopressin-induced deterioration in cardiac function. This should be further investigated and might be at least partially related to a similar effect of both arginine vasopressin and levosimendan on myocardial adenosine triphosphate-sensitive potassium channels.^{35,36} Accordingly, our study demonstrated that arginine vasopressin induced an afterload-independent deterioration of cardiac function. This is further supported by the lack of deterioration in cardiac function with norepinephrine alone despite a similar increase in mean arterial pressure in our endotoxemic rabbits. This difference between arginine vasopressin

and norepinephrine might be related to a potential β -receptor agonist effect of norepinephrine³⁷ that was not described with arginine vasopressin. Of note, the norepinephrine-induced increase in glycemia was also likely related to a β -receptor agonist effect. Although troponin I was not altered by arginine vasopressin or norepinephrine during the study period, one cannot exclude a direct vasoconstrictive effects on coronary circulation in septic rabbits that was recently described with arginine vasopressin in isolated perfused normal rabbit hearts.³⁸ Whether the effect of arginine vasopressin on cardiac contractile function is related to reduced coronary perfusion or to a direct effect on cardiac myocytes should be investigated.

Our study further assessed effects of levosimendan, arginine vasopressin, and norepinephrine on renal macrocirculation and microcirculation. Data on the effects of levosimendan on renal function are few and remain controversial.^{21,39} Pagel *et al.*³⁹ showed that levosimendan increases renal medullary blood flow and decreases cortical blood flow in normal dogs, whereas Ordner *et al.*²¹ showed no change in renal blood flow in a very early phase of sepsis. Our study confirmed the absence of effects of levosimendan on both macrocirculation and microcirculation in septic animals despite a marked improvement in cardiac output. The consequences of levosimendan on renal function, including creatinine clearance and glomerular filtration, should be further investigated in septic animals. Our study further compared the effects of arginine vasopressin and norepinephrine on renal hemodynamics. As shown in figure 4, despite a similar and modest increase in mean arterial pressure, arginine vasopressin and norepinephrine had divergent effects on renal medullary blood flow: Norepinephrine alone markedly increased renal medullary circulation, whereas arginine vasopressin alone rapidly deteriorated it. Norepinephrine is increasingly used as first-line therapy to restore mean arterial pressure in septic patients. Increasing recent evidences demonstrated no harmful and even beneficial effects of norepinephrine or phenylephrine on renal function in animals⁴⁰ and in humans during sepsis.^{41,42} Our study further shows that such improvement in renal function with no change in renal blood flow could be related to a change in renal blood flow partition toward medullary regions of the kidneys.

By contrast, arginine vasopressin worsened medullary renal blood flow, with no change in cortical renal blood flow, in our endotoxemic rabbits. Previous works showed that the vasoconstrictive effects of arginine vasopressin induce an increase in arterial blood pressure together with a decrease in both cortical and medullary blood flow in normal animals.^{43,44} Albert *et al.*³⁴ recently confirmed the latter in normal rabbits but more importantly showed that arginine vasopressin increased mean arterial blood pressure, renal blood flow velocity, mostly

in its diastolic component, and cortical renal blood flow in acutely endotoxemic rabbits, whereas medullary flow was unchanged. Furthermore, Sun *et al.*⁴⁵ showed an arginine vasopressin increase in urine output in the early phase of sepsis in sheep. Interestingly, our current study extends these data by showing that a low dose of arginine vasopressin that barely increased mean arterial blood pressure may decrease medullary blood flow in a more severe model of chronic endotoxemic animals. Further studies are needed to investigate whether the decrease in renal medullary flow observed in our study could be related to a direct vasoconstrictive effect of arginine vasopressin on renal medullary vessels or indirectly to the arginine vasopressin-induced decrease in cardiac output and how this impacts renal function and urine output.

A couple of limitations of the current study are noteworthy. Intravenous lipopolysaccharide does not fully represent a model of sepsis with a bacterial source. However, we previously showed^{3,13} that our model mimics the extent of depression and the time course of myocardial dysfunction previously described in septic patients.⁴⁶ Anesthesia did little to interfere with hemodynamic results presented above. The major results, namely lipopolysaccharide-induced impairment and the beneficial effect of levosimendan, both on myocardial function, were confirmed by echocardiography in slightly sedated, spontaneously breathing animals. Assessment of myocardial function in our study focused on systolic aortic velocity, ejection time, and maximal aortic acceleration using Doppler techniques as well as shortening fraction by echocardiography. Those parameters exclusively explore left ventricular systolic function with some dependency on load changes. Results should be confirmed with the pressure-volume loop technique that may even further explore myocardial diastolic parameters as relaxation and ventricular compliance.

In summary, our study explored cardiac and renal effects of new cardiovascular agents, including arginine vasopressin and levosimendan, with potential use in severe sepsis and septic shock in humans. Levosimendan improved, arginine vasopressin deteriorated, and norepinephrine had no effect on cardiac contractility in endotoxemic animals. In addition, norepinephrine and arginine vasopressin had opposite effects on renal medullary blood flow. Accordingly, levosimendan and norepinephrine both exert beneficial effects in endotoxemic animals and should be explored in human sepsis trials.

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References

1. Tavernier B, Garrigue D, Boule C, Vallet B, Adnet P: Myofilament calcium sensitivity is decreased in skinned cardiac fibres of endotoxin-treated rabbits. *Cardiovasc Res* 1998; 38:472-9
2. Tavernier B, Li J-M, El-Omar MM, Lanone S, Yang Z-K, Trayer I, Mebazaa A, Shah A: Cardiac contractile impairment associated with increased phosphorylation of troponin I in endotoxemic rats. *FASEB J* 2001; 15:294-6
3. Mebazaa A, De Keulenaer G, Paqueron X, Andries L, Ratajczak P, Lanone S, Frelin C, Longrois D, Payen D, Brutsaert D, Sys S: Activation of cardiac endothelium as a compensatory component in endotoxin-induced cardiomyopathy: Role of endothelin, prostaglandins, and nitric oxide. *Circulation* 2001; 104:3137-44
4. Dellinger RP, Carlet JM, Masur H, Gerlach H, Calandra T, Cohen J, Gea-Banacloche J, Keh D, Marshall JC, Parker MM, Ramsay G, Zimmerman JL, Vincent JL, Levy MM, Surviving Sepsis Campaign Management Guidelines Committee: Surviving Sepsis Campaign guidelines for management of severe sepsis and septic shock. *Crit Care Med* 2004; 32:858-73
5. Levy R, Deutschman C: Evaluating myocardial depression in sepsis. *Shock* 2004; 22:1-10
6. Ricard-Hibon A, Losser MR, Kong R, Beloucif S, Teisseire B, Payen D: Systemic pressure-flow reactivity to norepinephrine in rabbits: Impact of endotoxin and fluid loading. *Intensive Care Med* 1998; 24:959-66
7. Tavernier B, Makhotine O, Lebuffe G, Dupont J, Scherpereel P: Systolic pressure variation as a guide to fluid therapy in patients with sepsis-induced hypotension. *ANESTHESIOLOGY* 1998; 89:1313-21
8. Leone M, Vallet B, Teboul J, Mateo J, Bastien O, Martin C: Survey of the use of catecholamines by French physicians. *Intensive Care Med* 2004; 30:984-8
9. Martin C, Viviani X, Leone M, Thirion X: Effect of norepinephrine on the outcome of septic shock. *Crit Care Med* 2000; 28:2758-65
10. Patel BM, Chittock DR, Russel JA, Walley KR: Beneficial effects of short-term vasopressin infusion during severe septic shock. *ANESTHESIOLOGY* 2002; 96:576-82
11. Holmes CL, Patel BM, Russell JA, Walley KR: Physiology of vasopressin relevant to management of septic shock. *Chest* 2001; 120:989-1002
12. Holmes C, Walley K: Vasopressin in the ICU. *Curr Opin Crit Care* 2004; 10:442-8
13. Tavernier B, Mebazaa A, Mateo P, Sys S, Ventura-Clapier R, Veksler V: Phosphorylation-dependent alteration in myofilament Ca²⁺ sensitivity but normal mitochondrial function in septic heart. *Am J Respir Crit Care Med* 2001; 163:362-7
14. McBride B, White C: Acute decompensated heart failure: A contemporary approach to pharmacotherapeutic management. *Pharmacotherapy* 2003; 23:997-1020
15. Benlolo S, Lefoll C, Katchatouryan V, Payen D, Mebazaa A: Successful use of levosimendan in a patient with peripartum cardiomyopathy. *Anesth Analg* 2004; 98:822-4
16. Haikala H, Kaivola J, Nissinen E, Wall P, Levijoki J, Linden IB: Cardiac troponin C as a target protein for a novel calcium sensitizing drug, levosimendan. *J Mol Cell Cardiol* 1995; 27:1859-66
17. Pollesello P, Ovaska M, Kaivola J, Tilgmann C, Lundstrom K, Kalkkinen N, Ulmanen I, Nissinen E, Taskinen J: Binding of a new calcium sensitizer, levosimendan, to recombinant cardiac troponin C: A molecular modelling, fluorescence probe, and proton nuclear magnetic resonance study. *J Biol Chem* 1994; 269:28584-90
18. Hasenfuss G, Pieske B, Castell M, Kretschmann B, Maier LS, Just H: Influence of the novel inotropic agent levosimendan on isometric tension and calcium cycling in failing human myocardium. *Circulation* 1998; 98:2141-7
19. Lancaster M, Cook S: The effects of levosimendan on [Ca²⁺]_i in guinea-pig isolated ventricular myocytes. *Eur J Pharmacol* 1997; 339:97-100
20. Kaheinen P, Pollesello P, Levijoki J, Haikala H: Effects of levosimendan and milrinone on oxygen consumption in isolated guinea-pig heart. *J Cardiovasc Pharmacol* 2004; 43:555-61
21. Oldner A, Konrad D, Weitzberg E, Rudehill A, Rossi P, Waneczek M: Effects of levosimendan, a novel inotropic calcium-sensitizing drug, in experimental septic shock. *Crit Care Med* 2001; 29:2185-93
22. Behrends M, Peters J: The calcium sensitizer levosimendan attenuates endotoxin-evoked myocardial dysfunction in isolated guinea pig hearts. *Intensive Care Med* 2003; 29:1802-7
23. Abi Gerges N, Tavernier B, Mebazaa A, Faivre V, Paqueron X, Payen D, Fischmeister R, Méry P: Sequential changes in autonomic regulation of cardiac myocytes after in vivo endotoxin injection in rat. *Am J Respir Crit Care Med* 1999; 160:1196-204
24. Noble M, Trenchard D, Guz A: Left ventricular ejection in conscious dogs: I. Measurement and significance of blood from the left ventricle. *Circ Res* 1966; 19:139-47
25. Sabbah H, Khaja F, Brymer J, McFarland T, Albert D, Snyder J, Goldstein S, Stein P: Noninvasive evaluation of left ventricular performance based on peak aortic blood acceleration measured with a continuous-wave Doppler velocity meter. *Circulation* 1986; 74:323-9
26. Kivikko M, Antila S, Eha J, Lehtonen L, Pentikainen PJ: Pharmacodynamics and safety of a new calcium sensitizer, levosimendan, and its metabolites during an extended infusion in patients with severe heart failure. *J Clin Pharmacol* 2002; 42:43-51
27. Bichet DG, Arthus MF, Barjon JN, Lonergan M, Kortas C: Human platelet fraction arginine-vasopressin: Potential physiological role. *J Clin Invest* 1987; 79:881-7
28. Pinelli A, Trivulzio S, Tomasoni L, Brenna S, Bonacina E, Accinni R: Isoproterenol-induced myocardial infarction in rabbits: Protection by propranolol.

- lol or labetalol: A proposed non-invasive procedure. *Eur J Pharm Sci* 2004; 23:277-85
29. McBride B, White C: Levosimendan: Implications for clinicians. *J Clin Pharmacol* 2003; 43:1071-81
30. Stadlbauer KH, Wagner-Berger HG, Raedler C, Voelckel WG, Wenzel V, Krismer AC, Klima G, Rheinberger K, Nussbaumer W, Pressmar D, Lindner KH, Konigsrainer A: Vasopressin, but not fluid resuscitation, enhances survival in a liver trauma model with uncontrolled and otherwise lethal hemorrhagic shock in pigs. *ANESTHESIOLOGY* 2003; 98:699-704
31. Sharshar T, Blanchard A, Paillard M, Raphael J, Gajdos P, Annane D: Circulating vasopressin levels in septic shock. *Crit Care Med* 2003; 31:1752-8
32. Landry DW, Levin HR, Gallant EM, Seo S, D'Alessandro D, Oz MC, Oliver JA: Vasopressin pressor hypersensitivity in vasodilatory septic shock. *Crit Care Med* 1997; 25:1279-82
33. Malay MB, Ashton Jr, R Landry DW, Townsend RN: Low-dose vasopressin in the treatment of vasodilatory septic shock. *J Trauma* 1999; 47:699-703
34. Albert M, Losser M, Hayon D, Faivre V, Payen D: Systemic and renal macro- and microcirculatory responses to arginine vasopressin in endotoxic rabbits. *Crit Care Med* 2004; 32:1891-8
35. Landry D, Oliver J: The pathogenesis of vasodilatory shock. *N Engl J Med* 2001; 345:588-95
36. Kopustinskiene D, Pollesello P, Saris N: Potassium-specific effects of levosimendan on heart mitochondria. *Biochem Pharmacol* 2004; 68:807-12
37. Vincent JL: Vasoconstrictors, *Oxford Textbook of Critical Care*. Edited by Webb A, Shapiro M, Singer M, Suter P. Oxford, Oxford Medical Publications, 1999, pp 1282-5
38. Ouattara A, Landi M, Le Manach Y, Lecomte P, Leguen M, Boccard G, Coriat P, Riou B: Comparative cardiac effects of terlipressin, vasopressin, and norepinephrine on an isolated perfused rabbit heart. *ANESTHESIOLOGY* 2005; 102: 85-92
39. Pagel P, Hettrick D, Warltier D: Influence of levosimendan, pimobendan, and milrinone on the regional distribution of cardiac output in anesthetized dogs. *Br J Pharmacol* 1996; 119:609-15
40. Vane LA, Prough DS, Kinsky MA, Williams CA, Grady JJ, Kramer GC: Effects of different catecholamines on the dynamics of volume expansion of crystalloid infusion. *ANESTHESIOLOGY* 2004; 101:1136-44
41. Albanese J, Leone M, Garnier F, Bourgoin A, Antonini F, Martin C: Renal effects of norepinephrine in septic and nonseptic patients. *Chest* 2004; 126: 534-9
42. Di Giandomasso D, May CN, Bellomo R: Norepinephrine and vital organ blood flow during experimental hyperdynamic sepsis. *Intensive Care Med* 2003; 29:1774-81
43. Franchini KG, Cowley Jr AW: Sensitivity of the renal medullary circulation to plasma vasopressin. *Am J Physiol* 1996; 271:R647-53
44. Evans RG, Madden AC, Denton KM: Diversity of responses of renal cortical and medullary blood flow to vasoconstrictors in conscious rabbits. *Acta Physiol Scand* 2000; 169:297-308
45. Sun Q, Dimopoulos G, Nguyen D, Tu Z, Nagy N, Hoang A, Rogiers P, De Backer D, Vincent J: Low-dose vasopressin in the treatment of septic shock in sheep. *Am J Respir Crit Care Med* 2003; 168:481-6
46. Krishnagopalan S, Kumar A, Parrillo J, Kumar A: Myocardial dysfunction in the patient with sepsis. *Curr Opin Crit Care* 2002; 8:376-88