Comparison of Equipressor Doses of Norepinephrine, Epinephrine, and Phenylephrine on Septic Myocardial Dysfunction

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ABSTRACT

Background: Myocardial depression is a frequent event during septic shock and may mimic a cardiogenic shock state with decreased cardiac output. Nevertheless, data are scarce regarding the myocardial effects of vasopressors used to treat hypotension. In this study, the authors compared the effects of three commonly used vasopressors acting on different adrenergic receptors on myocardial function in a rodent model of septic shock, as explored with conductance catheter and positron emission tomography.

Methods: Septic shock was induced in rats by peritonitis. Eighteen hours after septic insult, vasopressors were titrated to increase mean arterial pressure by 20% compared with baseline values.

Results: We observed that peritonitis was associated with arterial hypotension and systolodiastolic dysfunction. Norepinephrine and epinephrine improved mean arterial pressure, cardiac output, and preload recruitable stroke work, a load-independent measure of systolic function, as well as diastolic function and ventriculoarterial coupling. Heart rate, myocardial oxygen consumption, and arrhythmia incidence were furthermore increased in the epinephrine group. Conversely, phenylephrine, a peripheral α-agonist, exhibited deleterious effects on systolodiastolic function and ventriculoarterial coupling. Conductance catheter and positron emission tomography yielded identical results with regard to myocardial function evolution under vasopressor treatment.

Conclusions: Phenylephrine, a drug without β-1 effects, was associated with decreased ventricular performance and ventriculoarterial uncoupling, whereas epinephrine and norepinephrine improved global hemodynamics and myocardial function in severely hypokinetic and hypotensive experimental septic shock. Nevertheless, epinephrine was associated with increased myocardial oxygen consumption. Thus, norepinephrine appears to be a more reliable and safer strategy as a first-line therapy in this particular setting.

What We Already Know about This Topic

- Myocardial dysfunction occurs during septic shock

What This Article Tells Us That Is New

- Norepinephrine and epinephrine improved global hemodynamics and myocardial function during experimental septic shock but epinephrine increased myocardial oxygen consumption, whereas phenylephrine decreased ventricular performance

CARDIOVASCULAR dysfunction is a major contributor in septic shock-induced mortality.[1] Septic shock is characterized by both an alteration in vascular tone[2] and a systolic and diastolic biventricular dysfunction.[3] In clinical practice, volume-resuscitated patients exhibit high cardiac output and low systemic resistance with myocardial depression despite the high output. The overall incidence of global left ventricular hypokinesia in patients with septic shock and no previous cardiac history is 60%, a value much higher than previously described.[4] In 10–20% of patients with septic shock, septic cardiomyopathy mimics a cardiogenic shock state with impaired cardiac output leading to death.[5] Cur-

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rent guidelines advocate an early use of vasopressor associated with fluid resuscitation for the treatment of arterial hypotension.[6] Nevertheless, and especially in the surgical setting, it is not clear whether vasopressors should be combined with inotropic drugs. Moreover, numerous adverse effects of catecholamines on heart function have been reported, ranging from tachycardia/tachyarrhythmia and myocardial stunning to necrosis and apoptosis.[7,8] Adverse cardiac effects of catecholamines are frequently dose-dependent and may counteract restoration of normal heart function. Moreover, vasopressors may induce a ventriculoarterial mismatch if myocardial performance does not match the effects on vasopressor tone.[9]

Norepinephrine, epinephrine, and phenylephrine are commonly used as vasopressor agents during septic shock.[6] Current evidence based on level A studies does not support recommendation of one vasopressor over another; indeed, norepinephrine, phenylephrine, and epinephrine can be used safely with similar survival outcomes.[9,10] When considering nonseptic cardiogenic shock or myocardial failure after cardiac surgery, both epinephrine[11] and norepinephrine[12] are recommended.

In general, for an equal increase in arterial pressure, cardiac output and oxygen delivery appear to be increased most with epinephrine, intermediately with norepinephrine, and less with phenylephrine.[6] Nevertheless, when looking at the effects of norepinephrine on heart contractile performance, it appears as efficient as epinephrine.[13,14] Experimental data on isolated myocardial heart tissues have shown that norepinephrine is inotropic and lusitropic at low and high concentrations whereas epinephrine increases inotropism at low concentration and decreases the latter at high concentration. This is explained by a differing action on β-2 receptors because both Gs and Gi pathways are stimulated by epinephrine, whereas only Gs is stimulated by norepinephrine.[15] In addition, phenylephrine and vasopressin have no positive effects on heart performance both in the healthy heart and in shock state.[6] Although the chronotropic, inotropic, and lusitropic effects of these drugs are well described in healthy hearts, sufficient data about their effects in septic cardiomyopathy have yet to be systematically evaluated to date.

In the current study, we used a rat model of septic shock explored 18 h after septic insult. Rats were volume resuscitated and treated with vasopressors. Myocardial function was explored with a conductance catheter placed in the left ventricle, which is the gold standard for myocardial function assessment,[16] and with micro positron emission tomography (PET), a new tool allowing the exploration of myocardial metabolism and myocardial function.[17]

We thus hypothesized that infusion of recommended vasopressors in septic shock induces different effects on intrinsic myocardial function and myocardial efficiency[18] and both explorative methods are complementary to fully characterize global hemodynamics and cardiac contractility during septic shock.

Materials and Methods

Animals

Male Wistar rats weighing 380–405 g were obtained from the Centre d'élevage Depré (St. Doulchard, France) and were acclimated at least 1 week before experimentation. All experiments were conducted in accordance with the National and European Institutes of Health guidelines for the use of laboratory animals and were approved by Nancy University. The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health.

Shock Model: Cecal Ligation and Puncture

Rats were anesthetized with isoflurane using 60% oxygen. A 2–3 cm ventral midline incision was performed. The cecum was ligated just distal to the ileocecal valve to avoid intestinal obstruction and punctured with a 21-gauge needle. The cecum was gently compressed to extrude cecal contents and returned to the abdomen. The incision was closed with suture. The left internal carotid artery and right external jugular vein were cannulated (tubing PE-50) under sterile conditions. In the sham-operated peritonitis group (Sham), the cecum was exposed, manipulated, and returned to the peritoneal cavity without being punctured.

All animals were resuscitated with 5 ml/100 g body weight of normal saline administered subcutaneously after completion of surgery and every 8 h thereafter.

Animals were subdivided into two groups in which hemodynamic parameters were continuously recorded. Myocardial performance was evaluated with the conductance catheter in the first group and using PET in the second group.

Eighteen hours after surgery, rats were anesthetized with isoflurane and randomized in five groups: Sham, cecal ligation and puncture (CLP), CLP-norepinephrine, CLP-epinephrine, and CLP-phenylephrine.

All animals were treated with volume expansion (5 ml saline/10 min). Vasopressors were infused in a dedicated venous line and titrated to maintain mean arterial pressure at $\pm$ 10% of sham values.

The same amount of fluid was infused in all rats. Two arterial blood samples were taken at baseline and at the end of the treatment to determine pH, arterial oxygen tension, partial pressure of alveolar carbon dioxide, glucose, and lactate levels.

Ventricular Contractility Measurement

Eighteen hours after peritonitis induction, isoflurane-anesthetized animals were mechanically ventilated after tracheostomy and instrumented for hemodynamic measurements. A MIKRO-Tip 2.0-French pressure-volume (P-V) conductance catheter (Millar Instruments, Houston, TX) was advanced into the left ventricle (LV) through the right carotid artery to measure LV P-V loops. Real-time volume conductance and pressure data were recorded using the EMKA pressure-volume conductance system (EMKA Technologies,

Paris, France) and LV volume was computed from conductance. This volume measurement was based on precalibration using a series of known cylindrical volumes as per manufacturer's instructions. Heart rate, end-systolic pressure, end-diastolic pressure, cardiac output, LV ejection fraction, end-systolic volume, end-diastolic volume, and stroke volume were measured at steady state. Maximum systolic pressure development divided by end-diastolic volume was also measured as an isovolumic phase index of ventricular contractility. Readings of conductance data yielded, in addition to LV volumes, maximum and minimum values of the first derivative of ventricular pressure over time (dP/dt_{max}, dP/dt_{min}), and the time constant of isovolumetric relaxation (τ). The pressure-volume relation was assessed by compression of the inferior vena cava, which temporarily changed preload and afterload. These data allowed computer calculation of load-independent variable of myocardial contractility such as prerecruitable stroke work (PRSW), which integrates the diastolic and systolic performance as the relation between stroke work and end-diastolic volume obtained during a vascular occlusion run. The total mechanical energy in the left ventricle (pressure-volume area) was also calculated as an estimate of myocardial oxygen consumption.[16] Cardiac power was calculated *post hoc* using the following formula: cardiac power = cardiac output * mean arterial pressure * 0.0022. Systemic vascular resistance (SVR) was calculated as the pressure drop (mean arterial pressure-central venous pressure) divided by cardiac output. The ratio of PRSW to SVR (PRSW/SVR) was used as an expression of the ventriculoarterial coupling ratio.[19] All indices were calculated using the IOX version 2.2.0 data analysis software (Millar Instruments).

Four measurements were performed: the first after 20 min of equilibration (baseline), the second after maximal volume loading and just before beginning vasopressor treatment, the third 30 min after the beginning of vasopressor infusion, and the fourth 60 min after the beginning of vasopressor infusion. Maximal volume loading was reached when additional fluids failed to further increase cardiac index.

Measurements (1,000/s) were acquired at baseline and periodically during a 60-min infusion of vasopressors, each administered in a separate cohort of animals.

PET Procedure

The PET investigation was planned to start at 18 h after surgery and was conducted using a previously detailed method.[17] Briefly, rats were maintained during anesthesia (isoflurane: 1.5–2.5%) and approximately 70 MBq of 18F-fluorodeoxyglucose was injected into the tail vein. Rats had received an oral premedication of 50 mg/kg acipimox, with half of this dose injected 1 h before 18F-fluorodeoxyglucose injection and the other half injected just before 18F-fluorodeoxyglucose injection.[17]

PET was recorded in list-mode 1 h after 18F-fluorodeoxyglucose injection and throughout the experiments using a dedicated small animal PET system (Inveon, Siemens, Knoxville, TN). The animals were under continuous anesthesia, and were positioned in the prone position and placed on a heating pad to maintain a body temperature within the normal range. They were connected to a standard electrocardiogram monitor by three electrodes placed on the inner surfaces of limb extremities. Carotid artery and right jugular vein were catheterized for mean arterial pressure monitoring and medication infusion. Cardiac images were reconstructed from several 5-min list-mode recording intervals and at different time points. At each time point, images were reconstructed with a three-dimensional ordered subset expectation maximization algorithm and with a voxel size of $0.8 \times 0.4 \times 0.4$ mm. Sixteen intervals were used for the electrocardiogram-triggered images, providing a temporal resolution of 11–15 ms. Reorientation along the left ventricular long axis was achieved with dedicated software provided by an e.soft station (Siemens). LV end-diastolic volume, LV end-systolic volume, and LV ejection fraction were obtained from the set of contiguous electrocardiogram-triggered short-axis slices with the Cedars-Sinai's Quantitative Gated SPECT (QGS) software (Cedars-Sinai, Los Angeles, CA). The peak ejection rate, a load-dependent index of LV systolic function, and the peak filling rate, a load-dependent index of diastolic function, were also calculated.

The average heart rate value during PET acquisition was extracted from the list-mode recording data. Systolic blood pressure was in addition recorded at each time point by the tail-cuff method (PowerLab, Ad Instruments, Mountain View, CA).

Tissue Adenine Nucleotide and Lactate Measurements

At the end of the experiments, with the animal still sedated, heart tissue samples were rapidly excised with stainless steel tongs, immediately frozen in liquid nitrogen, and stored at −80°C. The acid extraction procedure protocol was based on deproteinization by perchloric acid followed by neutralization with potassium hydroxide and centrifugation. After centrifugation, 100 μl of the supernatant were mixed with 1.0 M acetate buffer (pH 4.5) and 4.0 M 2-chloroacetoaldehyde solution and heated for 20 min at 80°C. Finally, 10 μl of the resulting sample was then used for high-performance liquid chromatography analysis with a Nova-Pak C18 column. Adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP) were simultaneously detected by a fluorescence detector.[20] In addition, the total adenine nucleotide pool (total adenine nucleotide pool = ATP + ADP + AMP), the ATP/ADP ratio, and the total energy charge (total energy charge = (ATP + 0.5 ADP)/(ATP + ADP + AMP)) were also calculated from the above data.

Heart Histopathology

At the end of the conductance catheter study, hearts were removed under isoflurane anesthesia and rinsed in phosphate-buffered solution. After embedding, all hearts were

Table 1. Effects of Cecal Ligature and Puncture on Myocardial Function Using the Conductance Catheter

	Sham (n = 6)	CLP (n = 6)	CLP NE (n = 6)	CLP E (n = 6)	CLP P (n = 6)
MAP (mmHg)	120 ± 5	76 ± 6*	83 ± 19*	86 ± 9*	79 ± 18*
HR (beats/min)	403 ± 8	369 ± 28	363 ± 30	373 ± 23	382 ± 36
CO (ml/min)	65 ± 7	41 ± 7*	46 ± 4*	47 ± 11*	43 ± 6*
LVEF (%)	48 ± 5	26 ± 4*	25 ± 2*	25 ± 5*	31 ± 7*
dP/dtmax (mmHg/min)	9,400 ± 400	5,975 ± 600*	6,007 ± 650*	5,931 ± 400*	6,367 ± 696*
PRSW (mmHg)	120 ± 8	81 ± 9*	78 ± 18*	82 ± 14*	74 ± 15*
PRSW/SVR (ml/min)	70 ± 9	53 ± 7*	52 ± 6*	54 ± 6*	50 ± 6*
dP/dtmin (mmHg/min)	−6,900 ± 460	−5,250 ± 420*	−5,130 ± 203*	−5,104 ± 704*	−5,412 ± 660*
τ (ms)	7.1 ± 0.2	7.8 ± 0.4	7.7 ± 0.3	7.8 ± 0.7	7.9 ± 0.7
LVEDP (mmHg)	8 ± 1.7	14 ± 1.3*	14 ± 1*	14 ± 0.3*	15 ± 2.4*
LVEDV (μl)	413 ± 61	324 ± 77	295 ± 62	388 ± 80	321 ± 29
CP (W)	18 ± 1.8	8.1 ± 1.2*	10 ± 2*	10 ± 3*	9 ± 1*
PVA (μl/mmHg)	66,580 ± 13,330	15,075 ± 2,250*	17,702 ± 4,895*	14,877 ± 6,133*	16,834 ± 6,758*

* $P < 0.05$ *vs.* sham; no difference was observed between CLP groups.

CLP = cecal ligature and puncture; CO = cardiac output; CP = cardiac power; dP/dtmax = maximum value of the first derivative of ventricular pressure over time; dP/dtmin = minimum value of the first derivative of ventricular pressure over time; E = epinephrine; HR = heart rate; LVEDP = left ventricular end-diastolic pressure; LVEDV = left ventricular end-diastolic volume; LVEF = left ventricular ejection fraction; MAP = mean arterial pressure; NE = norepinephrine; P = phenylephrine; PRSW= prerecruitable stroke work; PVA = pressure-volume area; SVR = systemic vascular resistance; τ = time constant of isovolumetric relaxation.

sectioned parallel to the atrioventricular groove in four slices. The third slice up from the apex of the heart was used for all histologic analyses, sectioned at a thickness of 7 μm, and stained with hematoxylin-eosin safran and nitro blue tetrazolium. The pathologist performing the section analyses was blinded to the origin of the samples.

Statistical Analysis

Data are reported as mean ± SD. Baseline values were compared using an unpaired, two-tailed Student *t* test. The difference between groups was tested using a two-way analysis of variance (repeated time measurements and drug as independent variables). A repeated-measures one-way analysis of variance was used to evaluate within-group differences. When the F value was statistically significant, a paired Student *t* test with the Bonferroni correction was used. A *P* value <0.05 was considered significant.

Results

Sixty-one rats underwent CLP. The overall mortality rate of the procedure before the beginning of the vasopressor infusion was 21%. When these rats were compared with sham animals, CLP was associated with arterial hypotension (110 ± 6 *vs.* 88 ± 9 mmHg), increased heart rate (404 ± 16 *vs.* 438 ± 43 bpm) and increased lactate level (0.8 ± 0.5 *vs.* 3.8 ± 2.0 mM) (table 1).

CLP Decreases Systolic and Diastolic Myocardial Function

Changes detected by conductance catheter readings confirmed that LV systolic function was impaired in CLP-18 h rats compared with sham animals (table 1). Both LV ejection fraction and cardiac output were decreased in animals with sepsis. LV maximal rate of pressure development (dP/dtmax) was significantly decreased than in sham-treated animals (5,975 ± 600 *vs.* 9,400 ± 400 mmHg/min, $P < 0.0001$). PRSW, a load-independent measure of systolic function, was significantly decreased in peritonitis than in sham-treated animals (81 ± 9 *vs.* 120 ± 8 mmHg, $P < 0.01$).

Furthermore, pressure–volume catheter readings obtained herein precisely detailed abnormal changes in LV diastolic function in peritonitis. LV end-diastolic pressure was increased (14 ± 1.3 *vs.* 8 ± 1.7 mmHg, $P < 0.0001$), whereas LV end-diastolic volume was decreased although not significantly (324 ± 77 *vs.* 413 ± 61 μl). The time constant of isovolumetric relaxation (τ) was not significantly different between sham and CLP animals.

Pressure-volume area, an index of myocardial oxygen consumption, was significantly decreased in CLP animals (15,075 ± 2,250 *vs.* 66,580 ± 13,330 μl/mmHg, $P < 0.01$).

CLP also increased SVR, although this increase was not matched by a concomitant increase in carbon monoxide thus leading to deterioration in PRSW/SVR indices, indicating a ventriculoarterial mismatch.

Conductance catheter results were confirmed by PET (see table 1, Supplemental Digital Content 1, http://links.lww.com/ALN/A829). Using PET, a significant decrease was observed in systolic function parameters such as LV ejection fraction (from 67 ± 7 to 52 ± 2%), cardiac output (from 118 ± 14 to 85 ± 12 ml/min) and peak ejection rate (from 58 ± 5 to 41 ± 4 ml/min) in CLP animals. An abnormal change in diastolic function was evidenced by a decrease in peak filling rate (from 63 ± 14 to 43 ± 6 ml/min) whereas LV end-diastolic volume and heart rate remained relatively unchanged.

No abnormal changes in cardiac morphology were observed in animals with sepsis. In particular, no ischemic or necrotic myocardial areas were characterized in the myocardium of either septic (see video, Supplemental Digital

Table 2. Comparative Effects of Norepinephrine, Epinephrine, and Phenylephrine on Septic Myocardial Dysfunction Using Conductance Catheter

Parameter	Group	Baseline	Post FR	T + 60 min
MAP (mmHg)	NE	83 ± 19	92 ± 23	115 ± 17*
	E	86 ± 9	90 ± 16	116 ± 13*
	PE	79 ± 18	82 ± 4	105 ± 5*
HR (beats/min)	NE	363 ± 30	353 ± 24	374 ± 30*
	E	373 ± 23	359 ± 28	430 ± 18†
	PE	382 ± 36	384 ± 41	360 ± 30
CO (ml/min)	NE	46 ± 4	60 ± 14	82 ± 8*
	E	47 ± 11	62 ± 16	79 ± 13*
	PE	43 ± 6	64 ± 17	34 ± 15†
LVEF (%)	NE	25 ± 2	32 ± 3	41 ± 3*
	E	25 ± 5.	32 ± 9	41 ± 6*
	PE	31 ± 7	35 ± 7	18 ± 7*
PRSW (mmHg)	NE	78 ± 18	75 ± 15	104 ± 45*
	E	82 ± 14	79 ± 12	114 ± 21*
	PE	74 ± 15	76 ± 16	51 ± 37†
PRSW/SVR (ml/min)	NE	52 ± 6	53 ± 6	70 ± 8
	E	54 ± 6	53 ± 7.1	79 ± 11
	PE	50 ± 6	50 ± 7	20 ± 4†
τ (ms)	NE	7.7 ± 0.3	8.2 ± 0.5	7.4 ± 0.5
	E	7.8 ± 0.7	7.7 ± 0.6	7.4 ± 0.7
	PE	7.9 ± 0.7	7.7 ± 0.5	6.4 ± 1.0†
LVEDP (mmHg)	NE	14 ± 1	12 ± 0.9	14 ± 1.6
	E	14 ± 0.3	15 ± 2.5	15 ± 2.9
	PE	15 ± 2.4	16 ± 1.4	17 ± 4.4
LVEDV (μl)	NE	295 ± 62	394 ± 44	480 ± 75*
	E	388 ± 80	467 ± 49	573 ± 63*
	PE	321 ± 29	505 ± 62	642 ± 94*
CP (W)	NE	10 ± 2	12 ± 2	18 ± 3*
	E	10 ± 3	14 ± 4	21 ± 4*
	PE	9 ± 1	14 ± 4	8 ± 4
PVA (μl/mmHg)	NE	17,702 ± 4,895	17,997 ± 5,523	24,859 ± 4,307*
	E	14,877 ± 6,133	13,236 ± 4,863	37,764 ± 7,666†
	PE	16,834 ± 6,758	16,555 ± 5,410	12,588 ± 5,296

* $P < 0.05$ *vs.* baseline, † $P < 0.05$ *vs.* baseline and other groups.

CO = cardiac output; CP = cardiac power; E = epinephrine; HR = heart rate; LVEF = left ventricular ejection fraction; LVEDP = left ventricular end-diastolic pressure; LVEDV = left ventricular end-diastolic volume; MAP = mean arterial pressure; NE = norepinephrine; PE = phenylephrine; Post FR = postfluid resuscitation; PRSW = prerecruitable stroke work; PVA = pressure-volume area; SVR = systemic vascular resistance; τ = time constant of isovolumetric relaxation.

Content 2, http://links.lww.com/ALN/A831, which is a characterization of left ventricular function in a septic animal using 18-FDG PET imaging) or sham-operated animals (see video, Supplemental Digital Content 3, http://links.lww.com/ALN/A832, which is a characterization of left ventricular function in a sham animal using 18-FDG PET imaging).

Conversely, ATP, ATP/ADP ratio, and total adenine nucleotide pool were significantly decreased in septic *versus* sham-operated animals (see table 2, Supplemental Digital Content 1, http://links.lww.com/ALN/A829).

Comparative Effects of Epinephrine, Norepinephrine, and Phenylephrine on Septic Cardiac Dysfunction

All of the investigated drugs significantly increased mean arterial pressure and heart rate (table 2). Mean doses of vasopressors were as follows: norepinephrine 1.8 ± 0.24 μg/kg/min, epinephrine 3.25 ± 0.4 μg/kg/min, and phenylephrine 6.1 ± 0.48 μg/kg/min. Cardiac output improved in epinephrine- and norepinephrine-treated rats whereas it decreased in phenylephrine-treated rats. Epinephrine and norepinephrine both increased dP/dt max (+43 ± 13% *vs.* +35 ± 5%, ns), LV ejection fraction (+38 ± 5% *vs.* +37 ± 10%, ns), PRSW (+38 ± 8% *vs.* +37% ± 10%, ns) and cardiac power (+56 ± 7% *vs.* +55 ± 11%, ns). On the other hand, phenylephrine either did not change or decreased these parameters (fig. 1). Epinephrine induced a greater increase in heart rate than norepinephrine. Sepsis-induced diastolic dysfunction did not change with epinephrine or norepinephrine, whereas phenylephrine had a deleterious effect on diastolic function as illustrated by a significant increase in left ventricular filling pressures and a decrease in τ. P-V area, an index of myocardial oxygen consumption, was significantly more increased in the epinephrine group than in the norepinephrine group (+170 ± 30% *vs.* +43 ± 10%, $P < 0.05$) (fig. 1). Similar results were also

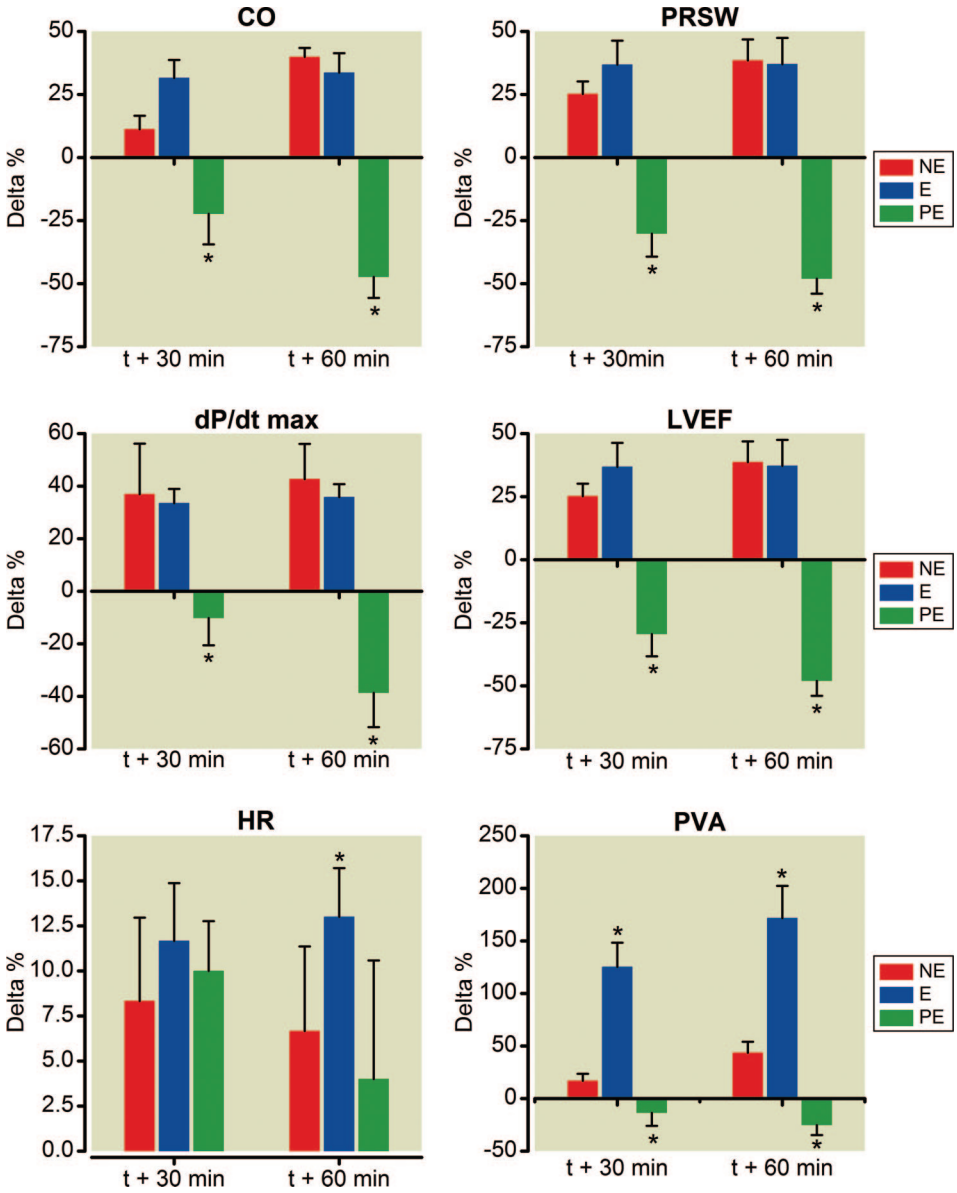

Fig. 1. Evolution of cardiac output (CO), prerecruitable stroke work (PRSW), dP/dtmax, left ventricular ejection fraction (LVEF), heart rate (HR), and pressure-volume area (PVA) using conductance catheter. E = epinephrine; NE = norepinephrine; PE = phenylephrine. Delta % = percentage of variation compared with baseline values; *t* + 30 min = 30 min after the beginning of vasopressor treatment; *t* + 60 min = 60 min after the beginning of vasopressor treatment. * $P < 0.05$ intergroup comparison.

observed with PET (see table 3, Supplemental Digital Content 1, http://links.lww.com/ALN/A829).

PRSW/SVR was normalized by norepinephrine (see video, Supplemental Digital Content 4, http://links.lww.com/ALN/A834, which is a characterization of LV function in a CLP animal treated with norepinephrine using 18-FDG PET imaging), and epinephrine (see video, Supplemental Digital Content 5, http://links.lww.com/ALN/A835, which is a characterization of LV function in a CLP animal treated with epinephrine using 18-FDG PET imaging), indicating favorable effects on ventriculoarterial coupling. Norepinephrine and epinephrine slightly increased SVR (+28 ± 5%, +23 ± 5%, $P > 0.05$). Phenylephrine, on the other hand, induced a profound ventriculoarterial mismatch with unfavorable effects on ventricular contractility associated with a pronounced increase in SVR (+380 ± 35%, $P < 0.05$) (see video, Supplemental Digital Content 6, http://links.lww.com/ALN/A836, which is a characterization of left ventricular function in a CLP animal treated with phenylephrine using 18-FDG PET imaging).

The incidence of arrhythmias was slightly increased by 23% in the epinephrine-treated group *versus* the norepinephrine- and phenylephrine-treated groups.

Histopathologic analyses showed no ischemic or necrotic lesions in rat hearts after catecholamine treatment (see figure, Supplemental Digital Content 7,

http://links.lww.com/ALN/A837). There was no improvement in ATP, ATP/ADP ratio, and total adenine nucleotide pool in treated groups (see table 2, Supplemental Digital Content 1, http://links.lww.com/ALN/A829).

Lactate concentration was significantly decreased in the norepinephrine group from 4.7 ± 2.6 to 3.4 ± 1.8 mM comparatively to an increase in epinephrine-treated group from 4.1 ± 1.3 to 6.5 ± 2.6 mM. Lactate levels did not change under phenylephrine treatment.

Discussion

The main findings of the current study are that, in a hypotensive and hypokinetic experimental septic shock model, both epinephrine and norepinephrine, administered alone, improved sepsis-induced vascular hyporesponsiveness, myocardial dysfunction, and ventriculoarterial coupling. On the other hand, phenylephrine, a pure vasopressor, was associated with a worsening of systemic and cardiac hemodynamics. As previously demonstrated, epinephrine treatment was associated with marked thermogenic effects that may limit its use in clinical practice.

Characterization of the Septic Model

One of the major limitations of previously used invasive and noninvasive approaches in the study of cardiac function in small animal models is that measured hemodynamic parameters were largely dependent on loading conditions. P-V analysis is a useful approach for examining intact chamber function independently of loading conditions. This analysis has been used extensively in large animal studies and in humans.[21] Recent advances in the development and validation of miniature P-V catheters has now made it possible to use this approach for studies in small animals.[22] Although P-V loop analysis is commonly used in mice, the combined P-V conductance catheter for rats has been introduced only recently, with very limited normative data being available. In the current study, we combined P-V catheter and micro PET, which allowed direct visualization of the moving heart as well as the assessment of both load-sensitive myocardial function and myocardial perfusion/metabolism. Both myocardial ATP content and ATP/ADP ratios were found to be decreased in CLP animals, indicating high-energy phosphate depletion and a dysfunction in mitochondrial respiratory chain phosphorylation.[23] These results, associated with the observed decrease in myocardial oxygen consumption and cardiac power, may suggest that septic cardiomyocytes reduce their energy requirements in an adaptive manner to prevent further damage and necrosis as previously hypothesized.[24]

Our study is the first to fully characterize global hemodynamics and cardiac function in a rat septic model using conductance catheter and PET with 18F-fluorodeoxyglucose. The studied model exhibited a typical pattern of septic shock with evidence of decreased vascular responsiveness and hypotension, lactic acidosis, and typical septic myocardial dysfunction with a marked systolodiastolic dysfunction and high-energy phosphate depletion. More importantly, and contrary to a PET study in an endotoxic model using labeled acetate,[25] we did not find any evidence of myocardial necrotic lesions using micro PET.

Furthermore, our septic shock model exhibited true LV dilatation after fluid resuscitation. When measured with the conductance catheter. Zanotti Cavazzoni *et al.*[26] had previously shown in a murine septic model that LV dilatation was a pattern of septic myocardial dysfunction and was associated with survival. Thus, the current model is clinically relevant and well suited to study both systolic and diastolic function and the effects of vasopressors on myocardial function during sepsis.

Phenylephrine Decreases Myocardial Performance

In the current study, phenylephrine likely induced an excessive increase in left ventricular afterload not compensated by positive effects on heart contractile force, which led to arterioventricular decoupling and severe heart failure as previously demonstrated in cardiogenic shock.[27] These results are not surprising when considering previous experimental studies. Indeed, similar data have been reported by Faivre *et al.*[28] These findings suggest that during septic shock, vasopressors without direct inotropic effects might be deleterious to myocardial performance.

Both Epinephrine and Norepinephrine Improve Myocardial Function and Arterioventricular Coupling

The differences observed between epinephrine and norepinephrine as previously found in septic[29] and cardiogenic shock[30] were that epinephrine induced higher heart rate, lactate level, and myocardial oxygen consumption. Moreover, epinephrine use was associated with increased supraventricular and/or ventricular arrhythmias. Thus, the well-known metabolic effects of epinephrine through extracardiac β-2 stimulation[31] led to a mandatory higher heart rate that may increase myocardial oxygen consumption and arrhythmia incidence. According to these results, norepinephrine may be preferred to epinephrine because the latter, by raising the basal metabolic rate, indirectly puts an extra burden on the heart, a property not shared by norepinephrine. In clinical practice, this may aggravate ischemia in patients with preexistent coronary lesions. Despite these potential deleterious effects, in our short-term study and using young previously healthy animals, there was no evidence of any vasopressor-induced cardiac ischemia or further depletion in high-energy phosphate.

Thus, when considering global hemodynamics and ventricular performance parameters, we did not find any differences between these two drug regimens. A major finding, however, is that both drugs significantly improved myocardial performance without the need for the adjunction of dobutamine. In clinical practice, adding dobutamine is advocated in case of low cardiac output but is often inefficient due to decreased β-receptor responsiveness.[32] Interestingly, Zausig *et*

al.[33] found that, in isolated septic rat hearts, epinephrine had the most favorable results with regard to cardiac efficiency when compared with dobutamine and levosimendan. Nevertheless, epinephrine is associated with a transient lactic acidosis, excessive tachycardia and arrhythmia, and increased myocardial oxygen consumption that may hamper its use, especially in patients at high risk of myocardial ischemia.

Load-sensitive versus *Load-independent Parameters to Estimate Myocardial Function*

We found that for a given arterial pressure level, load-sensitive parameters (ejection fraction, dP/dtmax, cardiac output), independently of the method used (conductance catheter or PET), and load-independent parameters (PRSW) yielded the same information when considering the evolution of ventricular performance under vasopressor effects. These data are of clinical importance because they allow the estimation of myocardial performance by using more simple tools such as echocardiographic parameters.

Limitations

The techniques used herein present some limitations. Even if conductance catheter remains the gold standard for determining cardiac inotropism and LV volume, accuracy of load-independent indices such as PRSW could be limited by the nonlinearity of *in situ* end systolic pressure volume relationship.[34] Furthermore, the inhomogeneity of the electric field in *in situ* hearts could underestimate cardiac volumes especially when there is a quick decrease in loading conditions (*e.g.*, occlusion of inferior vena cava).[35] However, in our rat study, the load variation range is limited and measurements in these conditions remain accurate in assessing cardiac function as previously published.[34]

PET in small animals is a powerful tool to provide noninvasive imaging information and to monitor cardiac function by allowing calculation of widely used indices. However, even if PET resolution is less than 1 mm in small animals, bias could occur for LV volume calculation related to image reconstruction and mathematical modeling.[36]

In the current study, the dose of vasopressor drug was adjusted *a priori* to fixed arterial pressure level, which provided multiple advantages over fixed doses. First, this approach took into account the variability in the pharmacodynamics of the adrenergic agents in animals with septic shock. Because the administration of adrenergic agents results in unpredictable plasma concentrations, these concentrations may not necessarily be correlated with pharmacologic effect.[2] Second, this method clearly reproduced clinical practice in which vasopressor dose was adjusted to predefined levels of arterial pressure (65–70 mmHg).[37] Because we wanted to challenge heart performance with vasopressors in the worst heart situation, we voluntarily used a severely hypokinetic model of septic shock. This might have exaggerated the deleterious effects of phenylephrine observed in the current study. Morelli *et al.*[38] found that there were no differences in terms of cardiopulmonary performance, global oxygen transport, and regional hemodynamics when phenylephrine was administered instead of norepinephrine in the initial hemodynamic support of septic shock. Recent data from Rehberg *et al.*[39] demonstrated that other vasopressors and inotrope combinations might be of interest, such as norepinephrine plus vasopressin plus levosimendan.

Nevertheless, our model mimics a frequent clinical situation (late peritonitis) and is clearly associated with vascular and myocardial dysfunction and certainly appears to be suitable for mechanistic studies.

Finally, the study was limited to the short-term effects of treatments and this timing did not take into account other effects of catecholamines such as immune effects.[40] Hence, it remains to be determined if the current results are applicable to more prolonged infusions.

Conclusions

Load–sensitive and load-independent myocardial parameters measured alternatively with conductance catheter or micro-PET were similarly modified under vasopressor treatment. Drug without β-1 effects such as phenylephrine was associated with a marked ventriculoarterial uncoupling and should be avoided in cases of sepsis-induced cardiac failure. This may be important, especially in situations in which hemodynamic monitoring is limited such as in the emergency or operating room. Conversely, epinephrine and norepinephrine improved global hemodynamics and myocardial function in severely hypokinetic and hypotensive experimental septic shock. Nevertheless, epinephrine was associated with increased myocardial oxygen consumption without altering heart structure, myocardial perfusion, and myocardial energetics. Therefore, norepinephrine thus appears to be a more reliable and safe strategy as a first-line therapy in this particular setting.

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