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Impaired heart rate regulation and depression of cardiac chronotropic and dromotropic function in polymicrobial sepsis

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Abstract

The scope of cardiac pathophysiology in sepsis has not been fully defined. Accordingly, we evaluated the effects of sepsis on heart rate (HR), HR variability, and conduction parameters in a murine model of sepsis. Electrocardiograms were recorded non-invasively from conscious mice before and after cecal ligation and puncture (CLP) or sham surgery. Responses of isolated atria to tyramine and isoproterenol were quantified to assess the functional state of sympathetic nerves and postjunctional sensitivity to adrenergic stimulation. CLP mice had lower HR compared to sham at 16–18 h post-surgery (Sham: 741±7 beats per min, CLP: 557±31 beats per min, n=6/group, P<0.001), and there was significant prolongation of the PR, QRS and QTc intervals. Slowing of HR and conduction developed within 4–6 h after CLP and were preceded by a decrease in HR variability. Treatment of CLP mice with isoproterenol (5 mg/kg, i.p.) at 25 h post-surgery failed to increase HR or decrease conduction intervals. The lack of *in vivo* response to isoproterenol cannot be attributed to hypothermia since robust chronotropic and inotropic responses to isoproterenol were evoked from isolated atria at 25 and 30° C. These findings demonstrate that impaired regulation of HR (*i.e.*, reduced HR variability) develops before the onset of overt cardiac rate and conduction changes in septic mice. Subsequent time-dependent decreases in HR and cardiac conduction can be attributed to hypothermia and would contribute to decreased cardiac output and organ perfusion. Since isolated atria from septic mice showed normal responsiveness to adrenergic stimulation, we conclude that impaired effectiveness of isoproterenol *in vivo* can be attributed to reversible effects of systemic factors on adrenergic receptors and/or post-receptor signaling.

Keywords

atrial contractility; atrial rate; cardiac conduction; heart rate; heart rate variability; isoproterenol; tyramine

Introduction

Cardiac dysfunction is recognized as a major complication of sepsis and is often associated with unfavorable outcomes (1-3). Adverse effects of sepsis on cardiac function can occur through several direct and indirect mechanisms that include volume depletion and decreased preload, disruption of autonomic neural control, and cardiomyopathy caused by proinflammatory mediators and activated immune cells (1, 4, 5).

Most clinical and experimental studies of cardiac function in sepsis have focused on evaluation of inotropic properties (2, 6-8). The presence of myocardial depression after adequate fluid resuscitation is characteristic of severe sepsis and indicative of intrinsic cardiac dysfunction. Furthermore, clinical studies have shown that septic patients with depressed left ventricular ejection fraction have a poor prognosis (1, 2, 9). While the etiology of septic cardiomyopathy is complex, proinflammatory cytokines clearly play an important role (1, 4, 9). Experimental studies suggest that these mediators can impair cardiac function directly through effects on cardiomyocytes and indirectly by disrupting autonomic regulation of the heart (1, 4, 5, 9).

Several recent clinical studies have focused on evaluating changes in cardiac chronotropic function in sepsis (10-12). This interest was undoubtedly sparked by a large body of data, which shows an association between decreased heart rate (HR) variability and diseases such as heart failure, hypertension and diabetes (13). Studies of neonates and adults have provided evidence that some HR variability measures decrease before the onset of sepsis (10, 12). Other clinical studies suggest that reduced HR variability might be associated with a poorer prognosis in septic patients (10, 11).

Many cardiovascular studies of endotoxic and septic shock in animal models have focused on evaluating mechanisms of inotropic dysfunction (6, 7). The use of anesthetic in some of these experiments precluded a precise evaluation of cardiac chronotropic function and HR variability. However, telemetric recording methods were applied in a recent study to evaluate the effects of endotoxin on HR and HR variability in conscious mice (14). These investigators reported that high dose endotoxin reduced several indices of HR variability in the mouse and found that these decreases correlated with increased levels of inflammatory cytokines in the blood. Similar studies with rats showed that endotoxin evoked prominent increases in HR and decreases in HR variability (15). The effects of sepsis on HR and HR variability have not been evaluated in conscious mice.

The primary goal of this study was to evaluate the effects of cecal ligation and puncture (CLP) induced sepsis on chronotropic and dromotropic function in the mouse. Electrocardiograms (ECGs) were recorded from conscious mice through footpad electrodes with the ECGenie system. Adrenergic responsiveness was evaluated *in vivo* using the same system and *in vitro* by measuring chronotropic and inotropic responses of isolated whole atria.

Materials and Methods

Animals

Adult, male C57BL/6 mice purchased from Harlan Laboratories were used for this study at 4 to 6 months of age. Animal protocols were approved by the East Tennessee State University Committee on Animal Care and conformed to guidelines of the National Institutes of Health as published in the *Guide for the Care and Use of Laboratory Animals* (Eight Edition, National Academy of Sciences, 2011).

Mouse model of sepsis

Mice underwent cecal ligation and puncture (CLP) as previously described to induce polymicrobial sepsis (16-18). Briefly, mice were anesthetized with isoflurane, and a midline incision was made in the anterior abdominal wall. The cecum was exposed, ligated distal to the ileocecal junction with 0 Ethicon suture, and punctured once with a 20 gauge needle in an avascular region near the distal end. Feces were extruded through the hole, and the abdomen was closed in two layers. Sham mice received the same surgery without CLP. Sham and CLP mice received a subcutaneous injection of resuscitation fluid (1 ml of lactated Ringers) after surgery. This protocol for CLP causes severe disease in C57BL/6 mice, but a majority of animals survive for 2 days.

Recording of electrocardiogram

ECG recordings were obtained non-invasively using the ECGenie apparatus (Mouse Specifics, Inc., Boston, MA) as described previously (19, 20). Briefly, individual mice were removed from their home cage and placed on the elevated recording platform of the apparatus a few minutes prior to each data collection time to allow for acclimation. ECG signals were then acquired through disposable footpad electrodes located in the floor of a recording platform (21). Approximately 200 raw ECG signals were analyzed per mouse at each time point using e-MOUSE software (Mouse Specifics, Inc., Boston, MA), which employs processing algorithms for peak detection, digital filtering, and correction of baseline for motion artifacts. Heart rate (HR) was determined from R-R intervals, and HR variability was calculated as the mean difference in sequential HRs for the entire set of ECG signals analyzed (21, 22). The software also determined cardiac intervals (i.e., PR, QRT, and QTc), which were within the normal ranges for our unoperated and sham mice (21-23).

Measurement of body temperature

Rectal temperature was measured in some experiments to identify and quantify hypothermia. This was accomplished using a MicroTherma 2T hand held thermometer and a 0.75 in isolated rectal probe (Braintree Scientific, Braintree, MA).

Evaluation of isolated atrial function

CLP, sham or naive control mice were deeply anesthetized with 5% isoflurane and euthanized by decapitation. Hearts were removed and placed in cold, oxygenated (95% O₂, 5% CO₂) Krebs-Ringer bicarbonate buffer (pH 7.35 to 7.4) of the following composition (in mM): 120 NaCl, 4.7 KCl, 1.2 KH₂PO₄, 25 NaHCO₃, 2.5 CaCl₂, 1.2 MgCl₂, and 11.1 D-

glucose. The entire atrium was dissected intact from the ventricles and suspended vertically in an isolated tissue bath. For this purpose, each atrial appendage was impaled with a small metal hook (#28 trout hooks) attached to 5-0 suture. The left atrium was anchored to the bottom of a vertical support rod in the 15 mL tissue bath, and the right atrium was attached to a 25-g force transducer (World Precision Instruments, Sarasota, FL) positioned above the bath. Buffer in the tissue bath was oxygenated continuously and maintained at either 25°, 30° or 37°C. Spontaneous atrial contractions were recorded at a resting tension of 0.3 to 0.5 g using a ML224 Bridge Amplifier (ADInstruments, Colorado Springs, CO), a PowerLab/8SP, and a computer running Chart software version 5.2. Concentration response data for tyramine and L-isoproterenol were collected in a non-cumulative manner and analyzed using Prism software version 6.0 (GraphPad Software, La Jolla, CA). Responses to tyramine and isoproterenol were measured in the same atria from sham and CLP mice. For these experiments, tyramine was evaluated first. The preparations were then washed extensively with buffer and given 30 min for return to baseline before measuring responses to isoproterenol. Responses to isoproterenol alone were evaluated at 25° and 30° C in control atria from normal mice.

Drugs

L-isoproterenol hydrochloride and tyramine hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO).

Statistical analysis

Statistical comparisons and graphing of data were accomplished using GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA). Group data are presented as the arithmetic mean $\pm$ SE (n). Statistical comparisons were made using a paired or unpaired t-test or repeated measures analysis of variance (ANOVA) as appropriate. The Tukey procedure or Sidak's multiple comparisons test was used for *post hoc* comparisons after ANOVA. A probability level of 0.05 or smaller was used to indicate statistical significance.

Results

Heart rate and cardiac conduction are reduced in mice with polymicrobial sepsis

For initial experiments, we evaluated the ECG at 16 -18 h post-surgery, a time when CLP mice show visual signs of illness (*i.e.*, reduced motor activity and poor grooming). Recording of ECGs with the ECGenie requires handling of the mice, which causes some activation of the sympathetic nervous system and a consequent elevation of baseline HR compared to the intrinsic HR or baseline rate determined by telemetry (14, 23, 24). Accordingly, baseline HRs were elevated before surgery and did not differ between experimental groups (750 ± 11 beats per min for sham and 747 ± 7 for CLP, $n=6$ per group; Fig. 1A). HR decreased significantly in septic mice when measured at 16-18 h after CLP, but sham surgery had no effect on HR (Fig. 1A). Analysis of other ECG parameters showed that the PR, QRS, and QTc intervals were prolonged significantly in CLP mice at 16 to 18 h after surgery, but these parameters were unaffected by sham surgery (Fig. 1B-D). Subsequent experiments showed that body temperature was reduced significantly in CLP

mice compared to shams at 16 h post-surgery ($36.9 \pm 0.2^{\circ}\text{C}$ for sham and $31.8 \pm 1.3^{\circ}\text{C}$ for CLP; $n=6$ per group, $P=0.003$, unpaired t-test)

Isolated whole atria from septic mice exhibit normal baseline function and responsiveness to directly and indirectly acting adrenergic agonists

Immediately after collection of ECG data from the preceding groups of mice, the atria were removed and evaluated functionally in isolated tissue baths. Neither spontaneous beating rates nor contractility differed between atria obtained from sham or septic mice (Table 1). Likewise, isolated atria from sham and septic mice showed no differences in chronotropic or inotropic responses to the indirectly-acting sympathomimetic, tyramine (Fig. 2A and 2B; -log EC50 values for chronotropic response: $5.133 \pm 0.201\text{M}$ for sham and $5.158 \pm 0.241\text{M}$ for CLP; -log EC50 values for inotropic response: $5.195 \pm 0.454\text{M}$ for sham and $4.749 \pm 0.468\text{M}$ for CLP), or the directly-acting sympathomimetic, L-isoproterenol (Fig. 3A and 3B; -log EC50 values for chronotropic response: $8.854 \pm 0.342\text{M}$ for sham and $8.552 \pm 0.369\text{M}$ for CLP; -EC50 values for inotropic response: $7.949 \pm 0.337\text{M}$ for sham and $7.758 \pm 0.181\text{M}$ for CLP).

Slowing of heart rate and cardiac conduction develop early after the induction of sepsis

Since HR and body temperature were depressed at 16 - 18 h post-CLP and ECG intervals were prolonged, we evaluated the onset of these changes in the next series of experiments. Serial ECGs and body temperatures were collected hourly for the first seven hours after sham and CLP surgery. Pre-surgery values for HR and other ECG parameters did not differ between groups, but significant suppression of cardiac rate (Fig. 4A) developed by 4 h after CLP surgery. Further decreases in HR occurred over time in the CLP mice, reaching an average of 526 ± 42 beat per min at 7h post-surgery compared to 759 ± 9 for the sham group ($n=7$ per group, Fig. 4A). This bradycardic response to sepsis was preceded by a significant reduction of HR variability, which was first evident at 1 h post-surgery (Fig. 4B). HR variability remained depressed at 7 h post-CLP and two earlier time points. Over the same interval, CLP mice showed a gradual reduction of body temperature, reaching an average of $32.6 \pm 0.6^{\circ}\text{C}$ at 7h post-surgery compared to 37.0 ± 0.2 for the sham group ($n=4$ per group, Fig. 4C). Smaller but significant decreases in temperature were detected at 1 and 3 h post-CLP. The decreases in HR were paralleled by increases in PR interval (Fig. 5A), QRS interval (Fig. 5B), and QTc interval (Fig. 5C) in septic mice. Increased PR interval suggests slowing of atrial conduction. QRS interval reflects ventricular depolarization, while the QTc interval includes depolarization and repolarization. Accordingly, increases in QTc were detected earlier after CLP and had a larger magnitude compared to QRS (Fig. 5B,C). PR, QRS, and QTc intervals for sham mice fell within the normal range reported by others (21-23) and did not vary over time (Fig. 5).

Acute treatment with isoproterenol does not reverse chronotropic or dromotropic dysfunction in septic mice

To further assess potential causes for impaired chronotropic and dromotropic function in conscious septic mice, we evaluated cardiac sensitivity of CLP mice to *in vivo* adrenergic stimulation with isoproterenol at an intraperitoneal dose of 5 mg/kg. Previous studies have

demonstrated that this dose or less causes a significant tachycardia in normal mice (21, 24-26). Serial ECGs were recorded from CLP mice at 25 h post-surgery before and after administration of isoproterenol. HR and cardiac conduction times at 25 h after CLP were slowed significantly compared to baseline, pre-surgery values (Fig. 6). However, treatment with isoproterenol failed to increase HR or conduction significantly at times up to 1 h after treatment (Fig. 6). Although temperature was not evaluated in this experiment, a subsequent evaluation of other sham and CLP mice at 24 h post-surgery showed a significant reduction of body temperature for CLP mice (Sham: $37.2 \pm 0.1^{\circ}\text{C}$, $n=4$; CLP: $30.4 \pm 1.8^{\circ}\text{C}$, $n=6$; $P=0.0156$).

Responses of isolated atria to isoproterenol are maintained under hypothermic conditions

Since CLP mice in our study developed a time-dependent hypothermia, as reported by others (27-29), we evaluated the effect temperature on baseline chronotropic and inotropic function of isolated whole atria and on their response to isoproterenol. For these experiments, isolated atria from normal control mice were maintained at 25 or 30° C instead of the normal temperature of 37° C. Baseline HR was reduced in a temperature-dependent manner, as expected, but the magnitude of atrial contractions was less affected (Table 1). Positive chronotropic and inotropic responses to isoproterenol persisted at both lower temperatures. In fact, concentration-response curves for both parameters were shifted to the left and maximum percent changes were increased at 30° C compared to sham or CLP atria evaluated at 37° C (Figs. 3 and 7; chronotropic response at 30° C: $-\log \text{EC}_{50} = 9.077 \pm 0.087 \text{ M}$, inotropic response at 30° C: $-\log \text{EC}_{50} = 8.107 \pm 0.382 \text{ M}$). Atrial rhythm remained stable at 30° C, but occasional arrhythmias developed at 25° C, preventing the collection of complete concentration-response data. However, we did evaluate responses to a maximally effective concentration of isoproterenol (10^{-6} M) and observed prominent increases in rate ($197 \pm 82\%$, $n=3$) and force of contraction ($101 \pm 21\%$, $n=3$), albeit with a somewhat slower onset than occurred at 30° C (several seconds versus immediate).

Discussion

The mouse model of sepsis induced by CLP is widely used in experimental studies, however, little is known about the impact of CLP sepsis on chronotropic and dromotropic functions of the heart. Our *in vivo* experiments establish that CLP causes a rapid decrease in HR variability and time-dependent reduction of HR and prolongation of ECG parameters of conduction in the mouse. The latter changes begin as early as 4 h after CLP and are well established after 24 h. Treatment of septic mice with isoproterenol at this time failed to increase HR or improve conduction parameters. However, isolated atria from septic mice exhibited normal baseline function and normal chronotropic and inotropic responses to the directly and indirectly acting sympathomimetics, isoproterenol and tyramine. Thus, impaired chronotropic and dromotropic responses of septic mice to isoproterenol must be due to systemic factors that reversibly impair these aspects of cardiac function and their responsiveness to adrenergic stimulation. Septic mice also develop a time-dependent hypothermia, which likely contributes to impaired cardiac function through direct cardiac and indirect neuronal mechanisms. However, chronotropic and inotropic responses of

isolated atria to isoproterenol persist even at 25° C, suggesting that reversible defects at the myocyte might contribute to impaired isoproterenol response *in vivo*.

Tachycardia due to reflex activation of the sympathetic nervous system is an early sign of sepsis in humans (1), and this response has been documented in some animal models of sepsis and endotoxemia (14, 30, 31). No tachycardic response was detected in the present study with mice following induction of sepsis by CLP. The absence of tachycardia may reflect the fact that HR was already elevated due to handling of the mice. Telemetric studies of endotoxemia in mice have shown that intraperitoneal injection of high-dose LPS increases HR from a baseline of around 550 beats per min to a peak of about 700 beats per min at 2 to 4 h after treatment (14), however, there are significant differences between endotoxemia and sepsis. Since baseline HRs were already above 700 beats per min in our experiments, reflecting significant basal sympathetic activation, further increases in rate due to sepsis were probably masked. Nevertheless, it is well documented that hypotension occurs as an early response to CLP in mice (7), and it is likely that this drop in blood pressure would have activated the baroreflex. Furthermore, previous studies of mice with polymicrobial sepsis have provided evidence for a rapid and prolonged activation of the sympathetic nervous system, since significant elevation of plasma catecholamines occurred from 0.5 to 20 h after CLP surgery (32). Our finding that significant bradycardia develops within 4 h after CLP suggests that reflex tachycardia must be short-lived or is outweighed by mechanisms that cause cardiac suppression.

Our experiments with isolated atria from CLP and sham mice demonstrate that neither cardiac sympathetic nerves nor beta-adrenergic receptor-mediated atrial responses are impaired irreversibly in septic mice. Positive chronotropic and inotropic responses to tyramine occur primarily through the release of norepinephrine from cardiac sympathetic nerves (33). Since neither the potency nor the efficacy of tyramine differed between atria from CLP and sham mice, *in vivo* bradycardia cannot be explained by a sympathetic nerve defect. It remains possible that sympathetic tone might be reduced in septic mice at >4 h after CLP. However, such changes could not explain bradycardia that occurs before 20 h post-CLP when sustained elevation of plasma catecholamines has been reported (32). The lack of chronotropic response to intraperitoneal injection of isoproterenol in septic mice suggests that the primary defect occurs at the heart. It is unclear from our studies whether the lack of response to isoproterenol *in vivo* is due to receptor desensitization and/or some post-receptor defect (1). In either case, the defect must be rapidly reversible, since isolated atria from septic mice exhibit normal responsiveness to isoproterenol.

Several studies have shown that hypothermia can occur in mice after CLP (27-29), and decreased body temperature undoubtedly contributed to the cardiac suppression observed in our study (34-36). Previous experiments with rats have shown that controlled reduction of body temperature to 25° C decreases HR, prolongs conduction intervals, and eliminates the baroreflex (35). However, in accord with our results, little or no reduction in HR occurred at temperatures above 34° C (36). While hypothermia can explain the time-dependent slowing of HR and cardiac conduction, it cannot explain the lack of *in vivo* chronotropic response to isoproterenol at 25 h post-CLP surgery. Our experiments with isolated mouse atria showed that baseline HR was reduced significantly at 25 and 30° C, but responses to isoproterenol

remained robust, and the potency of isoproterenol for evoking positive chronotropic and inotropic responses actually increased at 30° C compared to 37° C. Previous studies with isolated perfused dog atria showed that chronotropic and inotropic responses to isoproterenol persisted at 25° C but at a reduced amplitude (37). The lowest average body temperature in the present study was 30° C at 24 h post-CLP.

Attenuated cardiac response to adrenergic stimulation has been reported in patients with severe sepsis and suggested as a contributing factor to sepsis-induced cardiac dysfunction (1). Impaired adrenergic responsiveness has been attributed to down-regulation of β -adrenergic receptors, suppression of post-receptor signaling pathways by cytokines and nitric oxide, and oxidative degradation of catecholamines (1). Previous studies have shown that treatment of spontaneously beating neonatal cardiac myocytes with inflammatory cytokines (e.g. IL-6) blocks positive chronotropic responses to isoproterenol (38). Since circulating levels of inflammatory cytokines are elevated in mice with CLP-induced sepsis (27, 29, 39), it is possible that effects from these mediators prevented positive chronotropic and dromotropic responses to isoproterenol in our septic mice. Isolation of the atria and frequent washing with normal buffer would provide time and environmental changes that support recovery of responses to isoproterenol.

There was a striking reduction of HR variability within the first hour after CLP in our study, suggesting that autonomic regulation of HR is rapidly altered early in sepsis before major changes in body temperature have occurred. This conclusion is supported by recent telemetric experiments, which evaluated HR and HR variability parameters in conscious mice treated with endotoxin or TNF α (14). Both treatments caused a rapid reduction in multiple indices of HR variability. Other investigators have reported that administration of interleukin-6, by intraperitoneal or intracerebroventricular injection, likewise decreases HR variability, although these experiments were performed in anesthetized mice (40). Several clinical studies have documented that HR variability can be suppressed in patients with sepsis and other diseases with systemic inflammation (10). It is noteworthy that the reduction of HR variability in our study occurred well in advance of decreases in HR or the prolongation of ECG conduction intervals. Likewise, decreased HR variability occurred prior to times reported for peak elevation of inflammatory cytokines in the blood after CLP in mice (27, 29, 39). The early onset of decreased HR variability that we observed in the murine model of sepsis is similar to results obtained in recent HR variability studies of patients in the emergency department (10-12, 41). These investigators established that changes in HR variability occur early during the course of acute illness and suggested that reduction of HR variability may predict patients at high risk for developing sepsis.

In summary, this study provides the first evidence that cardiac chronotropic and dromotropic functions become impaired rapidly in conscious mice with sepsis. Hypothermia contributes to these changes, but it is likely that impaired response of the heart to catecholamines also plays a role. Decreased HR would contribute substantially to reduction of cardiac output in septic mice and thereby have a major negative impact on organ perfusion. Overt decreases in heart rate in CLP mice were preceded by a marked reduction of HR variability, which appeared to be independent of hypothermia. This finding parallels recent clinical evidence, and supports the concept that reduced HR variability could have value in early diagnosis of

sepsis. We also found that isoproterenol treatment failed to increase HR or improve cardiac conduction in septic mice, indicating the presence of severe adrenergic dysfunction. This finding parallels reports of impaired inotropic response to adrenergic stimulation in patients with sepsis. Since isolated atria from septic mice had normal responsiveness to isoproterenol, we conclude that impaired adrenergic signaling *in vivo* was due to systemic factors (*e.g.*, elevated catecholamines and cytokines) and might be reversed if exposure to these factors is reduced or eliminated soon enough.

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Fig. 1. Sepsis decreases heart rate and cardiac conduction in conscious mice

ECGs were recorded non-invasively and analyzed with eMouse software to determine heart rates (A) and PR (B), QRS (C), and QTc (D) intervals. Values are the mean $\pm$ SE (n=6 per group). # P <0.05 or ## P <0.005 compared to corresponding pre-surgery value (paired t-test).

Fig. 2. Sepsis does not affect the response of isolated atria to the indirectly-acting adrenergic agonist tyramine

Concentration-response curves are shown for the positive chronotropic (A) and inotropic (B) effects of tyramine in isolated atria from sham and CLP mice. Values are the mean $\pm$ SE ($n=5$ for sham and $n=6$ for CLP groups). Two-way ANOVA with repeated measures showed significant effects of concentration ($P<0.001$, A and B) but no effect of treatment or interaction between treatment and concentration ($P>0.05$ Sham versus CLP, A and B).

Fig. 3. Sepsis does not affect the response of isolated atria to the directly-acting adrenergic agonist isoproterenol

Concentration-response curves are shown for the positive chronotropic (A) and inotropic (B) effects of isoproterenol in isolated atria from sham and CLP mice. Values are the mean $\pm$ SE ($n=5$ for sham and $n=6$ for CLP groups). Two-way ANOVA with repeated measures showed significant effects of concentration ($P<0.001$, A and B) but no effect of treatment or interaction between treatment and concentration ($P>0.05$ Sham versus CLP, A and B).

Fig. 4. Heart rate, heart rate variability and body temperature decrease rapidly after the induction of sepsis

Graphs show the time course for early changes in heart rate (A), heart rate variability (B) and body temperature (C) after sham and CLP surgery. Values are the mean $\pm$ SE (n=7 per group for A and B, n=4 for C). For each parameter, no differences were detected between groups prior to surgery ($P>0.05$). Post-surgical values for each parameter were evaluated by two-way ANOVA with repeated measures. A. Heart rate (HR): two-way ANOVA with repeated measures showed significant effects of treatment ($F_{1,12}=28.13$, $P<0.0002$), time

($F_{6,72}=23.7$, $P<0.0001$), and a treatment-time interaction ($F_{6,72}=21.51$, $P<0.0001$). B. Heart rate variability (HRV): two-way ANOVA with repeated measures showed a significant effect of treatment only ($F_{1,12}=33.79$, $P<0.0001$). C. Body temperature: two-way ANOVA with repeated measures showed significant effects of treatment ($F_{1,6}=125.7$, $P<0.0001$), time ($F_{6,36}=13.14$, $P<0.0001$), and a treatment-time interaction ($F_{6,36}=15.97$, $P<0.0001$). Sidak's multiple comparisons test was used to identify differences between sham and CLP groups at each post-surgical time. # $P<0.05$, ** $P<0.01$, ## $P<0.005$, ### $P<0.0005$, *** $P<0.0001$.

Fig. 5. Cardiac conduction decreases rapidly after the induction of sepsis

Graphs show the time course for early changes in PR (A), QRS (B), and QTc (C) intervals after sham and CLP surgery. Values are the mean $\pm$ SE (n=7 per group). For each parameter, no differences were detected between groups prior to surgery ($P>0.05$). Post-surgical values for each parameter were evaluated by two-way ANOVA with repeated measures. A. PR interval: two-way ANOVA with repeated measures showed significant effects of treatment ($F_{1,12}=9.880$, $P<0.01$), time ($F_{6,72}=5.731$, $P<0.0001$), and a treatment-time interaction ($F_{6,72}=8.062$, $P<0.0001$). B. QRS interval: two-way ANOVA with repeated measures

showed a significant effect of time ($F_{6,72}=7.185$, $P<0.0001$) and a treatment-time interaction ($F_{6,72}=4.949$, $P<0.001$), but the treatment effect alone was not significant ($F_{1,12}=3.827$, $P=0.0741$). C. QTc interval: two-way ANOVA with repeated measures showed significant effects of treatment ($F_{1,12}=39.88$, $P<0.0001$), time ($F_{6,72}=10.97$, $P<0.0001$), and a treatment-time interaction ($F_{6,72}=9.206$, $P<0.0001$). Sidak's multiple comparisons test was used to identify differences between sham and CLP groups at each post-surgical time. # $P<0.05$, ** $P<0.01$, *** $P<0.0001$.

Fig. 6. Isoproterenol has no effect on heart rate or conduction intervals of conscious mice at 25 hours after CLP

Graphs show heart rate (A) and PR (B), QRS (C), and QTc (D) intervals in septic mice before and after the intraperitoneal injection of isoproterenol. Values are the mean $\pm$ SE (n=4 per group). One-way ANOVA with repeated measures was performed on each data set and, in each case, showed a significant effect of time. Post hoc testing showed that all values after baseline were different from the baseline value but not different from each other.

Differences compared to baseline are indicated in the figure (# $P<0.05$, * $P<0.01$, ### $P<0.05$, ** $P<0.001$, and *** $P<0.0001$).

Fig. 7. Isolated atria show robust rate and contractile response to isoproterenol at reduced incubation temperature

Graphs show concentration-response curves for the positive chronotropic (A) and inotropic (B) effects of isoproterenol in control atria maintained at 30° C. Values are the mean $\pm$ SE (n=3). The $-\log EC_{50}$ values for the chronotropic and inotropic responses were: 9.077 ± 0.087 and 8.107 ± 0.382 M, respectively.

TABLE 1

Baseline beating rate and contractile force are normal in atria from septic mice but reduced at lower incubation temperature.

Group	Rate (beats per min)	Contractile force (mg)
Sham, 37°C	311 ± 14 (5)	312 ± 15 (5)
CLP, 37°C	300 ± 11 (6)	295 ± 34 (6)
Control, 30°C	182 ± 21 (3)*	231 ± 39 (3)
Control, 25°C	107 ± 22 (3)*	186 ± 42 (3)

Values are mean ± SE (n)

* P<0.05 vs. CLP and Sham