Cellular Mechanisms of Endotoxin-Induced Myocardial Depression in Rabbits

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We investigated the mechanisms by which endotoxic shock induces intrinsic myocardial depression by studying cardiac myocytes isolated from 10 anesthetized instrumented rabbits given 172±42 (mean±SD) μg/kg IV endotoxin. Left ventricular (LV) depression developed 4±1 hours after endotoxin administration, with a 15±4% increase in LV internal end-systolic diameter, measured with sonomicrometers at a matched LV end-systolic pressure of 65±10 mm Hg. Normal LV pressure, arterial Po2, and pH were maintained to minimize confounding effects of ischemia, hypoxia, and acidosis. Cardiac myocytes from endotoxin-exposed rabbits had less unloaded cell shortening and lower peak rates of cell shortening (−dL/dt) and lengthening (+dL/dt) at [Ca2+] levels ranging from 0.5 to 16 mM when compared with myocytes isolated from normal rabbits or rabbits undergoing an identical protocol but without exposure to endotoxin. At 2 mM [Ca2+], cell shortening was depressed by ≈25% because of a decrease in action potential duration (207±70 versus 375±66 milliseconds). In contrast, there was only mild impairment of sarcoplasmic reticulum (SR) function. When myocytes were restimulated after rest periods of 4 to 480 seconds, the decrement in cell shortening (rest decay), peak −dL/dt and peak +dL/dt, and the recovery from rest decay were similar in myocytes from endotoxin-treated and normal rabbits. There was a greater decrement in cell shortening in the second beat of postrest recovery in myocytes from endotoxin-treated rabbits than in normal myocytes. This was partly due to a 12% decrement in action potential duration with rest decay, which did not occur in normal myocytes. The SR Ca2+ content assessed by contractures in 10 mM caffeine was similar in the two groups. We conclude that endotoxic shock produces a LV depression in vivo that persists in isolated myocytes studied in vitro. This intrinsic myocardial depression is largely related to endotoxin-mediated sarcolemmal alterations, which shorten action potential duration, and is not due to alterations in SR function. (Circulation Research 1993;73:125-134)

KEY WORDS • endotoxin • excitation-contraction coupling • action potential • sarcoplasmic reticulum • myocytes

The development of left ventricular (LV) depression in endotoxic shock may contribute to hemodynamic instability and refractory hypotension.1 It is not clear if LV function is impaired because of intrinsic myocardial depression or if this is secondary to the acidosis, hypotension, hypoxia, and/or circulating myocardial depressant factors that accompany septic shock. Although function is depressed in isolated heart and cardiac muscle preparations from animals with endotoxic shock,2 this does not necessarily indicate direct damage from endotoxin-mediated pathways but may represent less specific effects of sustained hypoperfusion, hypoxia, and/or acidosis. Furthermore, ventricular dilation may develop in endotoxemia.3-5 Although the mechanisms are unknown, ventricular dilation may produce structural alterations (eg, in the extracellular collagen matrix) that would remain present in isolated heart and cardiac muscle preparations, resulting in altered loading conditions and indirect impairment of myocardial function.

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The goal of this study was to determine if endotoxic shock induces intrinsic myocardial dysfunction. We studied cardiac myocytes isolated from animals exposed to endotoxin. We documented LV depression in vivo by measuring a rightward shift in the end-systolic pressure-diameter relation (ESPDR). We vigorously maintained LV pressure, arterial Po2, and pH within a physiological range and isolated cardiac myocytes before the development of refractory hypotension and acidosis. This isolated myocyte model permitted us to study the effects of endotoxic shock on intrinsic myocardial function without the confounding effects of ischemia, hypoxia, and acidosis and in the absence of neurohumoral effects or circulating myocardial depressant factors. We used this isolated myocyte model to examine cellular mechanisms for myocardial depression in endotoxic shock. Excitation-contraction coupling was impaired in myocytes isolated from endotoxin-treated animals. Unloaded cell shortening and lengthening were depressed over a wide [Ca2+] range. This was related to marked shortening of the action potential duration but only subtle alterations in sarcoplasmic reticulum (SR) function.

Materials and Methods

Intact Animal Studies

Animal preparation. New Zealand White rabbits were sedated with intramuscular ketamine (33 mg/kg) and
xyazine (5 mg/kg) (Schein Inc) to shave the chest, neck, and femoral areas. An intravenous line was started in an ear vein, and the rabbits were given a bolus of 10 mg/kg morphine sulfate intravenously. A tracheostomy was performed, and the animals were intubated and then placed on a mechanical respirator (Harvard Apparatus, South Natick, Mass). Anesthesia was maintained with 60% nitrous oxide and 40% oxygen gas and a continuous intravenous infusion of morphine sulfate (3 to 5 mg/kg per hour) and ketamine (2 to 5 mg/kg per hour) through a Harvard infusion pump. A median sternotomy was performed, and a pericardial cradle was created to expose the heart. A high-fidelity 3F micromanometer (model SPC-330, Millar Instruments, Houston, Tex) was introduced into the LV through an apical stab wound to measure LV pressure. A fluid-filled polyvinyl tubing was inserted into a femoral artery and attached to a Gould P23ID pressure transducer to monitor arterial blood pressure. The inferior vena cava was isolated, and a suture was loosely placed around it to facilitate transient occlusion of venous return. An electrocardiogram was monitored with standard surface leads placed into the chest wall.

LV external minor axis diameter was measured with a pair of piezoelectric crystals (3 to 4 mm diameter) sutured onto the anterior and posterior epicardial surfaces. A third crystal (1×2 mm) was tunneled at a 30° to 45° angle into the subendocardium just opposite the diameter crystal on the anterior wall to measure anterior wall thickness. Dimensions were measured with a sonomicrometer amplifier that had been custom-modified to measure very short (eg, 3 mm) distances (Triton Electronics, San Diego, Calif).

Experimental protocol. All measurements were obtained at end expiration with respirations temporarily suspended. Recordings were made during transient occlusion of the inferior vena cava to measure the ESPDR. After control measurements were recorded, rabbits were given an intravenous bolus of 100 to 225 μg/kg endotoxin (Esherichia coli 055B5 LPS No, 8B, List Biological Laboratories Inc, Campbell, Calif; kindly provided by E. Ziegler, University of California, San Diego). The ESPDR was measured every 30 minutes after the administration of endotoxin. To minimize the confounding influence of myocardial ischemia, LV pressures were maintained with intravenous boluses of prewarmed (37°C) normal saline (0.9% NaCl) with or without 6% dextran. Arterial pH was monitored every 15 to 30 minutes. Acidosis was initially corrected by increasing minute ventilation (increased respiratory rate) and then infusing sodium bicarbonate as needed to maintain arterial pH between 7.35 and 7.45. Arterial PO2 was maintained above 80 mm Hg with supplemental oxygen gas. After LV depression was documented by a rightward or downward shift in the pressure-length relation in the ESPDR, the animals were killed, and the hearts were excised for myocyte isolation.

Rabbits used for normal myocyte isolation were sedated with intramuscular injections of 33 mg/kg ketamine and 5 mg/kg xylazine. Intravenous access was obtained through an ear vein, and rabbits were euthanized with 125 mg/kg sodium pentobarbital before excision of the heart.

We also performed “sham” experiments. The sham rabbits and endotoxin-treated rabbits were treated identically in terms of anesthesia, surgery, surgical instrumentation, and duration of study. Sham rabbits were given an intravenous bolus of saline rather than endotoxin. We partially occluded the vena cava to lower peak systolic pressure by 20 mm Hg for 5 minutes to mimic the transient hypotension produced by endotoxin. We transiently occluded the vena cava to measure contractility (ESPDR) every 30 minutes for 5 to 6 hours.

Data acquisition and analysis. The electrocardiogram, LV and femoral artery pressures, and LV external diameter and anterior wall thickness dimensions were recorded on-line using a series 80386 IBM compatible personal computer with a Data Translation analog to digital conversion board and AT CODAS (Akron, Ohio) software. Data were sampled at a frequency of 500 Hz. The LV internal diameter was calculated by subtracting twice the anterior wall thickness from the LV external diameter using ADVCODAS software. End systole was defined by the maximum ratio of LV pressure and LV internal diameter (maximal elastance) calculated using ADVCODAS software. The ESPDR data were fitted to a quadratic equation and solved for an LV end-systolic internal diameter at a matched LV end-systolic pressure (common for all time points).

Isolated Myocyte Studies

Solutions. All solutions were made and stored in plastic containers. Tyrode’s solution consisted of (mM) NaCl, 136; KCl, 5.4; MgCl2, 1; NaH2PO4, 0.33; glucose, 10; HEPES, 10; and CaCl2, 0 to 16. Tyrode’s solution without Na+ or Ca2+ (0 Na+—0 Ca2+ Tyrode’s solution) contained (mM) LiCl, 140; KCl, 6; MgCl2, 1; HEPES, 10; and glucose, 10. Caffeine solution consisted of 10 mM caffeine (added as a solid) in 0 Na+—0 Ca2+ Tyrode’s solution. All solutions were titrated to a pH of 7.40 at 28°C.

Cell isolation. After the heart was excised, it was quickly rinsed in nominally Ca2+-free Tyrode’s solution to remove blood and then mounted on a Langendorff perfusion apparatus and perfused with prewarmed (37°C) nominally Ca2+-free Tyrode’s solution for 6 minutes. This was followed by perfusion for 30 to 40 minutes with 50 μM [Ca2+] Tyrode’s solution containing 0.75 mg/mL collagenase B (Lot BJA119N, Boehringer Mannheim Corp, Indianapolis, Ind) and 0.16 mg/mL protease (Sigma Chemical Co, St Louis, Mo). After enzymatic digestion, the LV was dissected and placed into a Petri dish. Tissue was gently dispersed, and the resulting myocyte suspension was passed through gauze to filter the remaining connective and fatty tissue. The cell suspension was rinsed with solutions containing progressively increasing levels of [Ca2+] to a final [Ca2+] of 2 mM contained in minimal essential media (MEM, Gibco, Grand Island, NY). Cells were stored in MEM, and media were changed every 2 to 3 hours. In all protocols, cells were studied within 12 hours (=60% studied within 6 hours) of cell isolation.

Isolated myocyte measurements. Cells were placed in a 0.5-mL glass chamber and visualized using an inverted microscope (Diaphot, Nikon). Cells were continuously perfused at 28°C at flow rates of 2 to 4 mL/min with 2 mM [Ca2+] Tyrode’s solution. Only rod-shaped cells with clear striations and no vacuoles were chosen for study. The long-axis dimension of the myocyte was
measured using a Panasonic GP-CD60 solid-state camera and video edge-detection system (Crescent Electronics, Sandy, Utah). Data were sampled at a frequency of 240 Hz and recorded onto the same computer system used for the intact animal measurements (see above). Cell-shortening tracings were differentiated using ADVCODAS software to obtain the peak rate of shortening (−dL/dt) and peak rate of lengthening (+dL/dt). The extent of shortening and time from stimulation to peak +dL/dt were also measured. In all cases, data from 5 to 10 consecutive steady-state beats were measured and then averaged.

Action potentials were recorded from isolated myocytes by connecting the output from a high-input impedance amplifier (model 1E-251, Warner Instrument Corp, Hamden, Conn) to the same computer system previously described. The resting membrane potential, maximal action potential amplitude, and action potential duration at 90% recovery of maximal amplitude (APD90) were calculated and averaged from three to five consecutive beats.

Myocyte function and [Ca2+] relation. To measure unloaded cell shortening over a range of [Ca2+] from 0.5 to 16 mM, each cell was attached in the middle using a micromanipulator (Narishige Scientific Instrument Laboratory, Tokyo, Japan) with a 5 μm diameter-tipped micropipette filled with nominally Ca2+-free Tyrode’s solution. Micropipettes were made from 1.5-mm thin-walled glass capillary tubing (World Precision Instruments Inc, Sarasota, Fl) using a horizontal pipette puller (model P-87, Sutter Instrument Co, Novato, Calif). Once attached to the micropipette, cells were lifted off the floor of the glass well and suspended in solution. This eliminated any external load due to cell adhesion to the glass. This was performed because it was unknown whether myocytes exposed to endotoxin would adhere to the glass floor of the chamber differentially than normal cells. Cells were stimulated to contract with platinum electrodes connected to a pulse generator (Accupulser A510, World Precision Instruments, Sarasota, Fl) at a rate of 0.5 Hz with a 5-millisecond pulse width and pulse amplitude set at 50% above threshold. Cells were stimulated for at least 8 to 10 minutes to ensure stability before recording steady-state cell shortening. Cell shortening was recorded at six different [Ca2+] values (0.5, 1, 2, 4, 8, and 16 mM) in a randomized order, except cells were exposed to the 16-mM concentration last because cells did not always survive at this concentration.

Rest decay protocol. Cells were perfused with 2 mM [Ca2+] Tyrode’s solution at 28°C and stimulated at a rate of 0.5 Hz to record steady-state shortening. Rest periods of 4, 8, 16, 32, 60, 120, 240, and 480 seconds were introduced, and stimulation was resumed after the rest period. Cells were stimulated for 4 to 5 minutes before recording steady-state shortening before each rest period. The decrease in cell shortening after rest (rest decay) and subsequent recovery of steady-state shortening (postrest recovery) were recorded for at least 2 minutes. The cell shortening during rest decay and postrest recovery were expressed as a percentage of steady-state cell shortening. The recovery data were fitted to an exponential equation to calculate the number of beats required for 50% recovery of steady-state shortening (T1/2 of rest recovery), measured from minimal cell shortening following rest.

Caffeine contractures. Myocytes were plated onto 0.5-mL glass chambers. The chambers were coated with 0.1 mg/mL poly-d-lysine (Sigma) for 10 to 20 seconds, rinsed with distilled water, allowed to dry, and then used within 1 to 2 hours. Cells were perfused with 2 mM [Ca2+] Tyrode’s solution at flow rates of 3 to 5 mL/min through a 1.5-mm glass capillary tube positioned within 0.5 to 1 mm away from the cell. After recording steady-state cell shortening during stimulation at 0.5 Hz, stimulation was stopped while continuing cell perfusion. Rapid solution changes were performed using a solenoid valve situated close to the glass chamber. After 45 seconds, the solution was changed to 0 Na+–0 Ca2+ Tyrode’s solution to remove sodium from the bath and minimize Na+-Ca2+ exchange. After another 15 seconds, the perfusion was rapidly changed to 10 mM caffeine in 0 Na+–0 Ca2+ Tyrode’s solution to induce a caffeine contracture. Administration of caffeine was considered sufficiently rapid if the cell shortening following exposure to caffeine developed at a rate equal or greater than the rate of steady-state cell-shortening twitch. The magnitude of the caffeine contractures was measured by the maximum amplitude of cell shortening relative to resting cell length, the ratio of the caffeine contractures to steady-state twitch contractions, and the augmentation of cell shortening from steady-state twitch shortening.

Action potential protocol. Microelectrodes with resistances between 25 and 50 MΩ were filled with 3 M KCl and attached to a high-input impedance amplifier. Stable impalpments were obtained by gently advancing the microelectrode at a 45° angle onto the cell surface. Impalpments were considered stable if cells could be stimulated continuously for 30 minutes with stable resting membrane potential, action potential duration, and morphology. Cells were stimulated through the microelectrode with square-wave pulses of 2-millisecond duration and amplitude set at 40% above threshold. Cells were perfused continuously with 2 mM [Ca2+] Tyrode’s solution at 28°C throughout the protocol. Cells were stimulated at a rate of 0.5 Hz for at least 8 to 10 minutes before recording steady-state action potentials. We also measured action potentials following a rest period of 60 seconds. In some cells, we measured action potentials at stimulation frequencies of 0.1 and 0.2 Hz.

Statistical analysis. We used a paired Student’s t test to analyze the change in end-systolic diameter at a matched LV pressure after endotoxin. An unpaired Student’s t test was used to compare normal myocytes with myocytes from endotoxin-treated animals for differences in APD90 and T1/2 postrest recovery. To compare cell shortening, peak +dL/dt, and peak −dL/dt over a range of [Ca2+] values and postrest beats 1 and 2 over a range of rest periods, we used analysis of variance with repeated measures and one grouping factor. We defined statistical significance at a value of P<.05. Results are presented as mean±SD.

Results

Left Ventricular Function

We gave a mean dose of 172±42 (mean±SD) μg/kg endotoxin intravenously to 10 rabbits. Rabbits devel-
Fig 1. Graph showing the typical effects of intravenous endotoxin (175 μg/kg) on left ventricular (LV) function in a rabbit. The LV end-systolic pressure (ESP) is plotted against the internal LV end-systolic diameter (ESD) to measure the end-systolic pressure-diameter relation (ESPDR). The ESPDR from different time periods following endotoxin administration are each represented by a different symbol. There was a progressive decrease in contractility as shown by the rightward shift in ESPDR. When measured at a common LV ESP of 70 mm Hg, there was a progressive increase in LV end-systolic internal diameter to 21% above the control value by 5 hours.

opened acidosis within 1 hour, with arterial pH reaching a nadir of 7.23±0.09, which was corrected within 18±10 minutes. The ESPDR returned to the control value after correcting the acidosis. Four of the 10 rabbits had a transient drop in blood pressure during the first hour of 27±10 mm Hg, which resolved over 5 to 10 minutes. All rabbits eventually required fluid therapy to maintain LV pressure and adjustment of arterial pH to correct acidosis. Once LV depression was documented, the heart was excised for myocyte isolation before the development of refractory hypotension and acidosis.

Endotoxin produced a time-dependent decrease in LV contractility. Fig 1 shows a typical example of the progressive rightward shift in ESPDR following the administration of endotoxin. In this example, the LV end-systolic diameter measured at a matched LV end-systolic pressure of 70 mm Hg increased by 21% 5 hours after the administration of endotoxin. The LV depression could also be described by the progressive decrease in LV end-systolic pressure of ~25% at a matched LV end-systolic diameter of 12 mm. In the group of 10 rabbits, systolic depression developed within 4±1 hours. Significant LV dysfunction was documented by a 15±4% increase in LV end-systolic internal diameter at a matched LV pressure of 65±10 mm Hg (P<.001).

Myocyte Shortening

Fig 2 shows that endotoxin depressed unloaded cell shortening. There was significant depression of cell shortening in myocytes from endotoxin-treated animals when compared with myocytes from normal animals (P<.0001). There was no significant interaction between the two curves (normal versus endotoxin) that describe the relation between cell shortening and [Ca²⁺]. Thus, for all [Ca²⁺] values, cell shortening was significantly lower in myocytes from endotoxin-treated rabbits. There were also significant decreases in peak rates of shortening (Δ-L/dt) (P<.0001) and lengthening (Δ+L/dt) (P<.0001) in myocytes from endotoxin-treated animals compared with normal myocytes (Fig 3). The differences in peak lengthening were significant at all [Ca²⁺] values; differences in peak shortening were significant only at 1, 4, 8, and 16 mM [Ca²⁺].

Fig 3. Graphs showing the peak rate of cell shortening (Δ-L/dt, panel A) and cell lengthening (Δ+L/dt, panel B) at [Ca²⁺] values ranging from 0.5 to 16 mM. The peak rates are normalized to the resting cell length (RCL) and are presented as mean±SD. There was a significant decrease in both the peak rate of cell shortening and cell lengthening in myocytes from endotoxin-treated rabbits as compared with normal myocytes (P<.0001). The difference in Δ-L/dt was significant at all [Ca²⁺] values; the difference in Δ+L/dt was significant at 1, 4, 8, and 16 mM [Ca²⁺].
To examine whether the impairment in peak lengthening rate was primarily related to the contractile dysfunction, we correlated peak lengthening rate with peak shortening for each cell at the six different \([\text{Ca}^{2+}]\) values. Typical examples of this correlation are shown in Fig 4A. There was a linear correlation between the peak rate of shortening and peak rate of lengthening in both normal myocytes and myocytes isolated from an endotoxin-treated rabbit. In this example, the correlation coefficients were 0.93 and 0.99, respectively. The mean correlation coefficients for the 15 normal myocytes and the 19 myocytes from endotoxin-treated rabbits were 0.88±0.09 and 0.94±0.06, respectively. Fig 4B shows linear correlation graphs for the two groups calculated from the mean slopes and intercepts. The intercepts of this relation were significantly different \((P=0.02)\) between the two groups. The slopes of this relation were only marginally different \((P=0.07)\) between the two groups. The arrow and dotted line mark the mean value of peak \(-\frac{\text{dL}}{\text{dt}}\) (−0.93±0.44 resting cell length per second) for normal cells at 2 mM \([\text{Ca}^{2+}]\). At this peak shortening rate, the peak lengthening rate was 5% lower for myocytes from endotoxin-treated rabbits than for normal myocytes (1.12 versus 1.18 resting cell lengths per second, respectively).

**Sham Experiments**

To evaluate whether the depression measured in myocytes from endotoxin-treated rabbits was due to the experimental preparation rather than endotoxin, we compared myocytes from two sham rabbits with both normal myocytes and myocytes isolated from rabbits exposed to endotoxin. Sham rabbits and endotoxin-treated rabbits were subjected to the same anesthesia and surgery, but sham rabbits received a bolus injection of saline rather than endotoxin. Although we produced a transient hypotension (peak LV pressures lowered by \(20\text{ mm Hg for 5 minutes}\)) early during the protocol, the sham rabbits were not exposed to acidosis or bicarbonate therapy and did not require fluid boluses to support arterial pressures.

There was no evidence of LV depression, with the ESPDR remaining stable in the sham rabbits after 4 to 6 hours. When compared with the myocytes from the sham rabbits, the myocytes isolated from endotoxin-treated rabbits still demonstrated significant decreases in cell shortening \((P<0.001)\), peak rate of cell shortening \((P<0.001)\), and peak rate of cell lengthening \((P=0.002)\) at all levels of \([\text{Ca}^{2+}]\) ranging from 0.5 to 16 mM. We found no significant difference in cell shortening and peak rates of shortening and lengthening between myocytes isolated from sham animals and normal myocytes (Fig 5). Since the myocytes obtained from sham animals behaved similarly to myocytes from normal animals (ie, rabbits without anesthesia, surgery, or endotoxin treatment), in subsequent protocols we used myocytes isolated from normal rabbits as our control group.

**Sarcoplasmic Reticulum Function**

The decrease in peak rate of lengthening (\(+\frac{\text{dL}}{\text{dt}}\)) in myocytes from endotoxin-treated rabbits suggests an impairment in relaxation processes. We evaluated SR function in intact myocytes by measuring rest decay and postrest recovery. Fig 6 shows the effects of a 60-second rest period on myocyte shortening. In normal myocytes and in myocytes obtained from endotoxin-treated rabbits, cell shortening was lower in the first beat after resuming stimulation (postrest or recovery beat 1). Cell shortening decreased even further in the second postrest beat ( recovery beat 2) and then gradually returned to steady-state values during 10 to 15 beats. The time course for this recovery was characterized by \(T_{1/2}\) (the difference between minimum postrest cell shortening, usually in recovery beat 2, and the steady-state value). \(T_{1/2}\) did not differ significantly between normal myocytes (\(2.0±0.6\) beats) and myocytes from endotoxin-treated rabbits (\(2.1±1.6\) beats, \(P=0.42)\).

We measured the decrease in cell shortening (rest decay) in postrest beats 1 and 2 as a function of the rest period. In both normal myocytes and myocytes from endotoxin-treated rabbits, rest decay was progressively greater as the rest period increased from 4 to 480 seconds. There was no significant difference in the rest decay of postrest beat 1 between normal myocytes and myocytes from endotoxin-treated animals \((P=0.28)\). However, there was a significantly greater decrease in cell shortening in postrest beat 2 in cells from endotoxin-treated rabbits compared with normal cells \((P=0.007)\) as shown in Fig 7A. The decrement in cell shortening reached a plateau after rest periods of 100 to 200 seconds. This plateau for the rest decay of beat 2 was lower in myocytes from endotoxin-treated rabbits than in normal myocytes \((P=0.06)\). In addition, there were significantly greater decreases in the peak rates of cell...
shortening (-dL/dt) and lengthening (+dL/dt) in myocytes from endotoxin-treated rabbits than in normal myocytes (P=0.02 in Figs 7B and 7C, respectively).

Caffeine Contractures

We assessed SR Ca\(^{2+}\) content indirectly by measuring caffeine contractures in normal myocytes and myocytes from endotoxin-treated rabbits. This was performed in 0 Na\(^+\)-0 Ca\(^{2+}\) Tyrode’s solution to minimize sarcolemmal Na\(^+\)-Ca\(^{2+}\) exchange. We performed caffeine contractures after a 60-second rest period to determine whether the rate of SR Ca\(^{2+}\) leak differed between normal myocytes and myocytes from endotoxin-treated rabbits. Fig 8 shows a typical caffeine contracture superimposed on the steady-state cell twitch. The cell shortening during the caffeine contracture developed as rapidly as the cell shortening during the steady-state twitch contraction. Table 1 summarizes the results of the caffeine contractures. There was no significant difference between 11 normal myocytes and 16 myocytes from endotoxin-treated rabbits in the amplitude of the caffeine contractures (P=0.34), the ratio of the caffeine contractures to steady-state cell twitch contractures

FIG 6. Graph showing the postrest recovery of myocyte shortening for normal myocytes and myocytes from endotoxin-treated rabbits after a 60-second rest period. The myocyte shortening (mean±SD), expressed as a fraction of the steady-state (% SS) value, is shown for each beat after resuming cell stimulation at 0.5 Hz: after 60 seconds of quiescence. (P=0.32), or the increase in cell shortening during caffeine contractures relative to steady-state cell twitch contractions (P=0.66).

FIG 7. Graphs showing rest decay data (mean±SD) for the second recovery beat, when stimulations are resumed after rest periods ranging from 4 to 480 seconds. Data are presented as a fraction of the steady-state value for cell shortening in panel A, peak rate of cell shortening (−dL/dt) in panel B, and peak rate of cell lengthening (+dL/dt) in panel C. In all three panels, data are presented as a percentage of the steady-state control value (before the rest period). There was significantly greater rest decay in myocytes from endotoxin-treated rabbits than in normal myocytes for cell shortening (P=0.007), peak −dL/dt (P=0.02), and peak +dL/dt (P=0.02).
FIG 8. Time course of a typical steady-state cell twitch (A) superimposed on the caffeine contracture (B) produced in the same cell by application of 10 mM caffeine in 0 Na\textsuperscript{+}–0 Ca\textsuperscript{2+} Tyrode’s solution after a 60-second rest period.

**Action Potentials**

We compared action potentials obtained from 13 normal myocytes and 10 myocytes from endotoxin-treated rabbits. Fig 9 shows typical action potentials from a normal myocyte and a myocyte isolated from an endotoxin-treated rabbit. The APD\(_{90}\) of these two action potentials are representative of the mean APD\(_{90}\) values for each group. Table 2 summarizes action potential data from all myocytes. The resting membrane potential and action potential amplitude did not differ between normal myocytes and cells from endotoxin-treated animals. However, the APD\(_{90}\) was 45% shorter in cells from endotoxin-treated rabbits than in normal cells (207 versus 375 milliseconds, respectively; \(P<.0001\)).

We also evaluated changes in APD\(_{90}\) in postrest beats 1 and 2 following a rest period of 60 seconds. Table 3 summarizes this data. In normal myocytes, there was no significant change in the APD\(_{90}\) of postrest beats 1 and 2 compared with the APD\(_{90}\) of the steady-state control beats. In the myocytes from endotoxin-treated rabbits, there was no significant change in APD\(_{90}\) of postrest beat 1, but there was a significant 12% decrease in APD\(_{90}\) in postrest beat 2 compared with the APD\(_{90}\) of the steady-state control beats. In 13 normal myocytes and 6 myocytes from endotoxin-treated rabbits, the action potential morphology did not change at low stimulation rates (0.1 and 0.2 Hz). There was no evidence of the “spike and dome” morphology in the action potential, characteristic of activation of the transient outward current.

**Discussion**

This study demonstrates that the cardiac depression induced by endotoxin documented in the in vivo environment remains present in isolated myocytes in the in vitro environment. Myocyte shortening was depressed in cells from endotoxin-treated rabbits over a wide range of [Ca\textsuperscript{2+}] values. The major mechanism for endotoxin-associated myocardial depression was a decrease in action potential duration, with only a subtle defect in SR function.

A number of studies have shown that endotoxemia decreases myocardial function in both in vivo\textsuperscript{1-8} and isolated heart and cardiac muscle preparations.\textsuperscript{2} However, it is not entirely clear from these studies whether the myocardial depression is primary or secondary. In the intact animal, elevated catecholamines and decreased systemic vascular resistance in endotoxic shock may mask myocardial depression. In contrast, acidosis, myocardial ischemia (secondary to hypotension and/or high-output failure), and circulating myocardial factors\textsuperscript{10,11} or cardiac depressant factors may contribute secondarily to the myocardial dysfunction in vivo. Ventricular dilation also occurs in endotoxic shock\textsuperscript{1,3,5} and can alter loading conditions, thereby contributing to myocardial depression. The mechanisms for ventricular dilation are not clear but may involve structural damage of the extracellular support matrix in the heart. Such changes could also contribute to myocardial depression found in isolated heart and cardiac muscle preparations. Therefore, we developed an isolated myocyte model to determine whether exposure to endotoxin in the intact rabbit depresses intrinsic myocardial function independent of external loading conditions and neurohumoral influences.

We documented myocardial depression in the whole animal by a rightward or downward shift in the LV ESPRD. This was documented by measuring an \(\approx15\%\) increase in LV end-systolic internal diameter at a

| Table 1. Caffeine Contractures After 60-Second Rest Periods |
|------------------|------------------|------------------|------------------|
|                  | n   | Control twitch (%) | Caffeine contracture (%) | Ratio (caffeine/twitch) | Increase (caffeine – twitch) |
| Normal           | 11  | 11.3±4.1           | 24.3±7.6               | 2.3±0.6               | 13.0±4.9                  |
| Endotoxin        | 16  | 9.9±2.6            | 23.0±7.1               | 2.4±0.6               | 13.0±5.9                  |

n, Number of cells. Values are mean±SD.
matched LV end-systolic pressure. During the experimental protocol, vigorous attempts were made to maintain normal LV pressure, arterial pH, and oxygen tension so that our results would not reflect the confounding influences of ischemia, acidosis, or hypoxia. Myocytes were isolated before the development of refractory hypotension or acidosis. Since our interventions primarily delayed rather than prevented severe acidosis and hypotension, our results likely reflect the myocardial depression effects of endotoxic shock rather than just endotoxemia.

We evaluated the effects of our experimental preparation using myocytes isolated from sham rabbits. Sham and endotoxin-treated rabbits underwent the same anesthesia, surgery, surgical instrumentation, and duration of surgery. In addition, we transiently lowered the blood pressure in the sham rabbits to mimic the transient hypotension observed in some of the endotoxin-treated rabbits. Since myocytes from endotoxin-treated rabbits were depressed compared with myocytes from sham rabbits, the myocyte depression cannot be attributed to the surgery or anesthesia. However, the sham rabbits were not exposed to acidosis or bicarbonate therapy as occurred in the endotoxin-treated rabbits.

Acidosis has negative inotropic effects by impairing excitation-contraction coupling at several sites, causing a decrease in transsarcolemmal Ca\(^{2+}\) currents, inhibition of SR Ca\(^{2+}\) release, decreased myofilament sensitivity to Ca\(^{2+}\), and decreased maximum force generation. These effects are related to intracellular acidosis, which develops more rapidly and is more severe with respiratory than metabolic acidosis. Although extracellular acidosis produces intracellular acidosis in isolated hearts, the intact heart appears more resistant to the development of intracellular acidosis. Nevertheless, in intact animals extracellular acidosis can deplete high-energy phosphates and impair energy metabolism in the absence of significant intracellular acidosis. These impairments are exacerbated by bicarbonate, which may explain why bicarbonate can worsen ventricular function when used to treat hypoxic lactic acidosis. In some studies, bicarbonate does not improve ventricular function nor intracellular acidosis.

The role of bicarbonate for treating acidosis remains controversial.

In our study, rabbits developed acidosis within 1 hour of exposure to endotoxin. This was rapidly corrected with adjustments in the respiratory rate and/or with bicarbonate infusions. The ESPDR did not change from control after correcting the acidosis, indicating that the transient metabolic acidosis and bicarbonate therapy did not produce sustained myocardial depression. Later episodes of acidosis developed more gradually, were milder, and were more readily reversible. Therefore, it is unlikely that transient episodes of acidosis or bicarbonate therapy contributed to the sustained myocardial depression observed in the intact ventricle after several hours. These factors are even less likely to contribute to the depression observed in isolated myocytes, which were studied in vitro conditions several hours later.

Differences in loading conditions and neurohumoral factors in the intact animal may make it difficult to correlate the degree of myocardial depression in the intact animal with that found in isolated myocytes. At a physiological [Ca\(^{2+}\)] of 2 mM, there was an \(\approx25\%\) decrease in cell shortening in myocytes from endotoxin-treated animals. Cell shortening was measured in the absence of external load with myocytes suspended in solution. The consequences of this degree of myocyte depression may be greater in the whole animal, where the myocytes must contract against a load, are stretched by ventricular dilation, and are exposed to circulating myocardial depressant factors.

The decrease in myocyte shortening and peak \(-dL/dt\) and \(+dL/dt\) in endotoxic shock were caused by impaired excitation-contraction coupling. Myocyte depression in endotoxic shock was associated with marked shortening of the action potential duration without affecting the resting membrane potential or the action potential amplitude. A 45% decrease in APD\(_{90}\) decreases the amount of Ca\(^{2+}\) entering through L-type Ca\(^{2+}\) channels (decreases the Ca\(^{2+}\) current) and hence reduces Ca\(^{2+}\)-induced SR Ca\(^{2+}\) release. The mechanism for reducing the APD\(_{90}\) in endotoxic shock is unknown but may involve direct damage to the L-type Ca\(^{2+}\) channel. Other possibilities include endotoxin-induced activation of outward K\(^{+}\) currents through the ATP-sensitive channel or delayed rectifier currents. Although activation of the transient outward current may shorten action potential duration, this mechanism seems less likely since we did not observe any action potential with a "spike and dome" morphology characteristic of the transient outward current, even at low stimulation frequencies.

Our data show reduced myocyte shortening over a wide range of [Ca\(^{2+}\)] values. This suggests that endotoxin may reduce myofilament sensitivity to Ca\(^{2+}\) and/or decrease the maximal Ca\(^{2+}\)-activated force. The contribution of these mechanisms to the myocardial depression in endotoxic shock deserve further study.

Prior studies have shown that endotoxic shock impairs SR Ca\(^{2+}\)-ATPase activity, which reduces Ca\(^{2+}\)-induced SR Ca\(^{2+}\) release, and alters Na\(^+\)-Ca\(^{2+}\) exchange. To determine the relevance of these subcellular findings in a more intact system, we evaluated SR function in the intact myocyte with rest decay and postrest recovery. When stimulation is resumed after a period of rest, the first postrest beat is decreased in amplitude from the

### Table 2. Action Potentials

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>RMP (mV)</th>
<th>Amplitude (mV)</th>
<th>APD(_{90}) (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>13</td>
<td>-90±3</td>
<td>129±4</td>
<td>375±64</td>
</tr>
<tr>
<td>Endotoxin</td>
<td>10</td>
<td>-92±5</td>
<td>130±5</td>
<td>207±70*</td>
</tr>
</tbody>
</table>

*n. Number of cells; APD\(_{90}\), action potential duration at 90% recovery. Values are mean±SD.

*P<.05 vs. corresponding normal value.

### Table 3. Action Potential Duration at 90% Recovery After 60-Second Rest Periods

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Control beat</th>
<th>Postrest beat 1</th>
<th>Postrest beat 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>11</td>
<td>377±91</td>
<td>402±172</td>
<td>455±195</td>
</tr>
<tr>
<td>Endotoxin</td>
<td>10</td>
<td>197±73</td>
<td>169±84</td>
<td>174±71*</td>
</tr>
</tbody>
</table>

*n. Number of cells; APD\(_{90}\), action potential duration at 90% recovery. Values are mean±SD.

*P<.02 vs. corresponding value for control beat.
steady-state value, a phenomenon known as rest decay. During rest periods, Ca\(^{2+}\) leaking out the SR can be extruded from the cell by Na\(^+-\)Ca\(^{2+}\) exchange. The decreased amplitude of postrest contractions reflects decreased SR Ca\(^{2+}\), which is determined by the balance between extrusion of cytosolic Ca\(^{2+}\) by Na\(^+-\)Ca\(^{2+}\) exchange and reuptake by the SR Ca\(^{2+}\)-ATPase. The recovery of cell shortening amplitude with resumption of stimulation (postrest recovery) reflects the SR reloading process with net Ca\(^{2+}\) gain from Ca\(^{2+}\) influx until SR Ca\(^{2+}\) levels and thus cell shortening amplitude return to steady-state values.

Our data show no difference in the decrement of shortening amplitude of the first postrest beat between the normal cells and cells from endotoxin-treated rabbits. This suggests that endotoxin does not significantly affect the rate of SR Ca\(^{2+}\) leak nor the rate of extrusion of leaked SR Ca\(^{2+}\) from the cell by Na\(^+-\)Ca\(^{2+}\) exchange. However, we cannot rule out the possibility that there is increased SR Ca\(^{2+}\) leak but that SR Ca\(^{2+}\) content is unchanged because of a concomitant decrease in Ca\(^{2+}\) extrusion by impaired Na\(^+-\)Ca\(^{2+}\) exchange. This seems unlikely since the capacity of the Na\(^+-\)Ca\(^{2+}\) exchange mechanism, even if impaired, should be sufficient to remove the small amounts of Ca\(^{2+}\) leaking from the SR, particularly after long rest periods. In any case, the SR Ca\(^{2+}\) content does not appear to be affected by endotoxic shock, as shown by the similarity in caffeine contractures between normal myocytes and myocytes from endotoxin-treated rabbits. We performed caffeine contractures under conditions that minimized Na\(^+-\)Ca\(^{2+}\) exchange.

In contrast to postrest beat 1, there was a significant decrease in the amplitude of postrest beat 2 in myocytes from endotoxin-treated rabbits compared with normal myocytes. This was associated with a 12% decrement in APD\(_{90}\) in postrest beat 2 compared with the steady-state APD\(_{90}\), which was already 45% shorter than the steady-state APD\(_{90}\) of normal myocytes. A decrease in the Ca\(^{2+}\) current (due to decreased APD\(_{90}\)) along with impairments in the SR Ca\(^{2+}\)-ATPase would lead to diminished SR Ca\(^{2+}\) content and decreased shortening amplitude of postrest beat 2 in myocytes from endotoxin-treated rabbits.

Endotoxic shock has been shown to impair SR Ca\(^{2+}\)-ATPase activity in subcellular fractions in some studies but not all studies. To evaluate the relevance of impaired SR Ca\(^{2+}\) uptake, we compared peak shortening rates to peak lengthening rates in intact myocytes. There were excellent linear correlations with mean r values of 0.9 for normal myocytes and myocytes isolated from endotoxin-treated rabbits. There was a significant difference in the intercepts and a marginally significant difference in slopes between the two groups. However, the consequences of these differences at physiological [Ca\(^{2+}\)] of 2 mM and a common peak shortening rate, there was only a 5% decrease in peak lengthening rate in myocytes from endotoxin-treated rabbits compared with normal myocytes. Most of the relaxation abnormalities in myocytes from endotoxin-treated rabbits were secondary to impairments in systolic function. Thus, the endotoxin-associated impairments in SR function found in subcellular fractions do not appear to be a major factor in the intact myocyte. Moreover, preservation of intact SR function in endotoxic shock is supported by the similar rates of postrest recovery (T\(_{1/2}\)) between myocytes from endotoxin-treated rabbits and normal myocytes.

In summary, we have found in an isolated myocyte model that endotoxic shock leads to intrinsic myocardial depression independent of alterations in loading conditions or extrinsic neurohumoral factors. Myocytes from endotoxin-treated rabbits have depressed shortening, peak rate of shortening, and peak rate of lengthening over a wide [Ca\(^{2+}\)] range. The myocyte depression is associated with a shorter action potential duration, although a decrease in myofilament sensitivity and/or maximum activated force may also contribute to the depressed function. Endotoxin does not affect SR function to a great degree in the intact myocyte, although there may be subtle defects in SR Ca\(^{2+}\) uptake. Impaired cellular relaxation was primarily related to depressed contractility. The intrinsic impairment in myocardial function by endotoxin may contribute to the refractory hypotension in endotoxic shock.

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Cellular mechanisms of endotoxin-induced myocardial depression in rabbits.
J Hung and W Y Lew

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