Reduced L-type calcium current in ventricular myocytes from endotoxemic guinea pigs

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Zhong, Juming, Tzyh-Chang Hwang, H. Richard Adams, and Leona J. Rubin. Reduced L-type calcium current in ventricular myocytes from endotoxemic guinea pigs. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H2312–H2324, 1997.—The circulatory response to gram-negative sepsis and its experimental counterpart, endotoxemia, includes a profound dysfunction in myocardial contractility that is resident to the myocyte and associated with reduced systolic free intracellular Ca2+ concentration ([Ca2+]i). We explored the possibility that decreased systolic [Ca2+]i in endotoxemic myocytes is correlated with reduced L-type Ca2+ current (I(Ca,L)). Ventricular myocytes were isolated from guinea pigs 4 h after an intraperitoneal injection of Escherichia coli lipopolysaccharide (LPS; 4 mg/kg). Membrane potentials and Ca2+ currents were measured using whole cell patch-clamp methods. The action potential duration of endotoxemic myocytes was significantly shorter than control values (time to 50% repolarization: LPS, 314 ± 23 ms; control, 519 ± 36 ms, P < 0.05). Correspondingly, endotoxemic myocytes demonstrated significantly reduced peak I(Ca,L) density (3.5 ± 0.2 pA/pF) and Ba2+ current (I(Ba)) density (7.3 ± 0.5 pA/pF) compared with respective values of control myocytes (I(Ca,L) density 6.1 ± 0.3 pA/pF, I(Ba) density 11.3 ± 0.8 pA/pF, P < 0.05). Endotoxemia-induced reduction in peak I(Ca,L) could not be attributed to alterations in current-voltage relationships, steady-state activation and inactivation, or recovery from inactivation. The β-adrenoceptor agonist isoproterenol, but not the Ca2+ channel activator BAY K 8644, reversed the LPS-induced reduction in peak I(Ca,L), cell contraction, and systolic [Ca2+]i. These data demonstrate that part of the host response to endotoxemia involves diminished sarcolemmal I(Ca,L) of ventricular myocytes.

MATERIALS AND METHODS

Animal model. Male albino guinea pigs weighing 300–400 g (Sasco, Omaha, NE) were injected intraperitoneally with Escherichia coli endotoxin (4 mg/kg, LPS; Sigma, St. Louis, MO) or an equivalent volume of sterile saline (control). Four hours after LPS injection, animals were injected intraperitoneally with 1,000 U of heparin and were killed by decapitation 15 min later. Hearts were removed quickly by thoracotomy and placed immediately into ice-cold Ca2+-free isolation media. Previous studies with this endotoxemic model demonstrate that at 4 h animals are normotensive, and hypotension and shock develop between 8 and 12 h (21). All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Missouri-Columbia.

Isolation of ventricular myocytes. Ventricular myocytes were isolated as previously described (26). Briefly, guinea pig hearts were perfused retrogradely through the aorta with Ca2+-free isolation media [Earle’s balanced salt solution (GIBCO) supplemented with (g/l) 0.35 MgCl2, 0.37 NaHCO3, 0.2 KH2PO4, 0.3 glucose, 1.1 glucose, 5.03 N-2-hydroxymethylpiperazine-N’-2-ethanesulfonic acid (HEPES), and 1 each of the essential amino acids and vitamins (GIBCO); pH 7.15–7.2; 280 mosM] at −37°C. Hearts were then perfused with Ca2+-free isolation media containing 0.08% collagenase B (Boehringer Mannheim, Indianapolis, IN) for 10 min at 37°C. Ventricles were then isolated, minced, and incubated in fresh isolation media containing 0.02% collagenase B and 50 μM Ca2+ for 3 min at 37°C. Myocytes were mechanically dispersed with a large-bore, fire-polished pipette, filtered through sterile gauze, and centrifuged at low speed (15 g).
After repeated rinses and centrifugations at gradually increasing Ca	extsuperscript{2+}, cells were resuspended in HEPES-buffered Krebs-Henseleit (HKH) solution consisting of (in mM) 118 NaCl, 4.7 KCl, 1.2 MgSO	extsubscript{4}, 1.2 KH	extsubscript{2}PO	extsubscript{4}, 2.0 CaCl	extsubscript{2}, 13.5 NaHCO	extsubscript{3}, 11 glucose, and 10 HEPES (pH 7.2–7.3) (26).

Whole cell patch-clamp technique. Action potential and sarcoplasmic Ca	extsuperscript{2+} current were recorded using whole cell single-electrode current-clamp and voltage-clamp modes, respectively, using an Axopatch-1D patch-clamp amplifier. Patch pipettes were pulled from borosilicate glass capillary tubes with a two-stage pul low and then fire-polished. Tip resistance pipettes were replaced with NaCl (10 mM) and KCl (100 mM), and the pH was adjusted to 7.2 with KOH. After establishment of the whole cell configuration, the amplifier was switched to current-clamp mode, resting membrane potential was recorded, and an action potential was elicited by a 12.5-ms current pulse (30% above threshold).

Cell shortening and [Ca	extsuperscript{2+}]i measurements. Ventricular myocytes freshly isolated from either LPS or control guinea pigs were incubated with the cell membrane-permeant form of fura 2 [fura 2-acetoxymethyl ester (AM), 2.5 µM, Molecular Probes] for 10 min at room temperature and were washed twice with HKH solution (without fura 2-AM). Cells were then resuspended in HKH solution for 1 h before subsequent measurement of [Ca	extsuperscript{2+}]i and cell contraction. Fura 2-AM was diluted from a 1 mM stock solution into HKH solution to a final concentration of 2.5 µM. The stock fura 2-AM solution was made in 100% dimethyl sulfoxide (DMSO). DMSO at 0.25% concentration had no effect on contractile function of either control or LPS myocytes.

Fura 2-loaded myocytes were placed in a cell microperfusion chamber mounted on a Nikon Diaphot inverted microscope and were perfused continuously with HKH solution. A rod-shaped myocyte with clear striations and sharp edges was localized by microscopic observation, and contractions were elicited by field stimulation at 0.5 Hz with two platinum electrodes mounted on either side of the superfusion chamber. Stimulation duration was 2 ms. When myocyte contraction achieved steady state in HKH solution (3 min), superfusion was then switched to HKH solution containing either 0.1 µM isoproterenol (Iso, Sigma) or 0.1 µM BAY K 8644 (Calbiochem, La Jolla, CA). Concentrations of Iso and BAY K 8644 were determined experimentally to elicit maximal effects on both LPS and control myocytes.

Cell contraction and [Ca	extsuperscript{2+}]i were measured simultaneously. Myocyte contraction was assessed by measuring cell length using a motion detector (Crescent Electronics, Ogden, UT). Intracellular fura 2 was excited by a collimated light beam from a 150-W Xe arc lamp passed via a liquid light guide through a circular interference filter wheel containing two 180° filter sections that provide 340- and 380-nm illumination. The cell was illuminated simultaneously with 600-nm light for display on the video monitor. Fura 2 fluorescence emission was diverted to a photomultiplier tube by means of a dichroic mirror and was demodulated into two separate analog signals corresponding to 340- and 380-nm excitations, which were fed into separate channels of an analog-to-digital converter (Scientific Solutions, Solon, OH). Before myocyte [Ca	extsuperscript{2+}]i was measured, background fluorescence of the measuring area without myocyte was set to zero. Myocyte autofluorescence was determined from a separate set of non-fura 2 loaded myocytes from the same heart preparations as the fura 2-loaded myocytes under identical measuring conditions or, in select cases, from myocytes before fura 2 loading in the microperfusion chambers. Cell length and fluorescence data were collected every 20 ms and analyzed using CODAS analysis software (DATAQ).

After data collection, fura 2 ratios were converted to [Ca	extsuperscript{2+}]i using the equation described by Grynkiewicz et al. (9)

\[
[Ca^{2+}]_i = K_d \times \beta \times \left( \frac{R - R_{min}}{R_{max} - R} \right)
\]

For fura 2, \(K_d = 500 \text{ nM}, \beta = 0.046 \text{ s}^{-1} \text{ mOD}^{-1} \text{ mM}^{-2}, R_{min} = 0.05, R_{max} = 0.21\).
where $R_{\text{min}}$ and $R_{\text{max}}$ are the fura 2 ratios in Ca$^{2+}$-free and Ca$^{2+}$-saturating conditions, respectively; $K_d$ is the effective dissociation constant; $\beta$ is the ratio for the 380-nm excitation spectrum intensity at Ca$^{2+}$-free and Ca$^{2+}$-saturating conditions; and $R$ is the measured fluorescence ratio (340/380 nm). For determination of $R_{\text{min}}$ and $R_{\text{max}}$, fura 2-AM-loaded myocytes were exposed to carbonyl cyanide p-(trifluoromethoxy)-phenylhydrazone (3 µM, Sigma) and 2-deoxyglucose (10 mM) for 20 min in a glucose-free HKH solution. Exposure to these chemicals allowed measurement of $R_{\text{min}}$ and $R_{\text{max}}$ during metabolic inhibition, which prevents hypercontracture on the introduction of high [Ca$^{2+}$] (2). Myocytes were then made permeable to Ca$^{2+}$ by treatment with ionomycin (50 µM, Calbiochem), and $R_{\text{min}}, R_{\text{max}},$ and $\beta$ were determined in HKH solutions containing 10 mM EGTA or 2 mM Ca$^{2+}$, respectively. In some cases, myocytes also were perfused with intermediate Ca$^{2+}$ concentrations using EGTA or Ca$^{2+}$ buffer solutions, and the $K_d$ values were calculated. Using these conditions, we measured $R_{\text{min}} = 0.279, R_{\text{max}} = 3.362,$ and $\beta = 6.864,$ and using the Grynkiewicz equation we calculate that the $K_d$ for fura 2 binding to Ca$^{2+}$ in our system was 488 nM.

RESULTS

Membrane potential of LPS and control myocytes. The LPS-induced reductions in both peak systolic [Ca$^{2+}$]$_i$ and cell contraction were correlated with a significant reduction in action potential duration, as shown with typical action potentials from control (Fig. 1A) and LPS (Fig. 1B) myocytes after establishment of whole cell patch-clamp configuration. Characteristics of action potentials for both groups of myocytes are summarized in Table 1. Neither the resting membrane potential nor membrane capacitance verified that peak $I_{\text{Ca,L}}$ values of LPS myocytes were significantly less than values of control myocytes. Figure 2, A and B, shows representative current tracings recorded from a control and an LPS myocyte, respectively. These currents show characteristics typical of L-type Ca$^{2+}$ currents and were blocked by 500 µM Cd$^{2+}$ (data not shown). Normalization of peak current amplitude to membrane capacitance verified that peak $I_{\text{Ca,L}}$ density in peak current by the end of a 20-min recording period (data not shown). To properly compare peak $I_{\text{Ca,L}}$ in two different cell populations (control and LPS), we measured peak $I_{\text{Ca,L}}$ at the same time period, and all measurements were completed within 15 min after establishment of the whole cell configuration.

Corresponding to shortened action potential duration, $I_{\text{Ca,L}}$ values of LPS myocytes were significantly less than values of control myocytes. Figure 2, A and B, shows representative current tracings recorded from a control and an LPS myocyte, respectively. These currents show characteristics typical of L-type Ca$^{2+}$ currents and were blocked by 500 µM Cd$^{2+}$ (data not shown). Normalization of peak current amplitude to membrane capacitance verified that peak $I_{\text{Ca,L}}$ density

Table 1. Action potential parameters for control and LPS myocytes

<table>
<thead>
<tr>
<th>Group</th>
<th>$n$</th>
<th>$E_m$, mV</th>
<th>Amplitude, mV</th>
<th>$\text{APD}_{50}$, ms</th>
<th>$\text{APD}_{90}$, ms</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>19 (7)</td>
<td>$-65 \pm 1.0$</td>
<td>$146 \pm 2.7$</td>
<td>$518 \pm 0.1$</td>
<td>$576 \pm 0.1$</td>
</tr>
<tr>
<td>LPS</td>
<td>18 (6)</td>
<td>$-64 \pm 1.3$</td>
<td>$143 \pm 4.4$</td>
<td>$314 \pm 0.1^*$</td>
<td>$384 \pm 0.1^*$</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = \text{no. of myocytes studied.}$ Nos. in parentheses are no. of animals. LPS, lipopolysaccharide; $E_m$, resting membrane potential; APD, action potential duration; $\text{APD}_{50}$ and $\text{APD}_{90}$, time to 50% and 90% repolarization, respectively. *Significantly different from control ($P < 0.05$).
of LPS myocytes was significantly lower than corresponding values from control myocytes (Fig. 2C). Reduction of \( I_{Ca,L} \) could not be explained by dissimilar sizes of control and LPS cells because membrane capacitance was not different between control (94.9 ± 1.9 pF) and LPS (92.5 ± 1.8 pF) myocytes. Although the peak \( I_{Ca,L} \) density was reduced in LPS myocytes, the possibility remained that total current throughout the 200-ms voltage pulse was similar between these two groups, which would be reflected as a decrease in the rate of \( I_{Ca,L} \) decay in LPS myocytes. The rate of \( I_{Ca,L} \) decay was best fit by a single exponential function in both control and LPS myocytes and was significantly faster in control myocytes compared with LPS (Fig. 2D). However, the residual current at the end of the 200-ms recording period was higher for control myocytes than for LPS myocytes, indicating that total charge movement was still greater for control myocytes (data not shown).

Effect of \( Ba^{2+} \) substitution on \( I_{Ca,L} \). L-type \( Ca^{2+} \) channels are known to be modulated by intracellular \( Ca^{2+} \) (3). Although the intracellular \( Ca^{2+} \) was buffered by 10 mM EGTA in the present experiments, a transient increase in local subsarcolemmal \( Ca^{2+} \) concentration during \( Ca^{2+} \) influx could affect \( Ca^{2+} \) channel activity (16). To eliminate the potential effect of subsarcolemmal \( Ca^{2+} \) on \( Ca^{2+} \) channel function, we measured currents with \( Ba^{2+} \) rather than \( Ca^{2+} \) in the bathing solution. For these experiments, currents were measured from myocytes that were first superfused with \( K^{+\text{-free}} \) Tyrode solution containing 1.8 mM \( CaCl_2 \), \( I_{Ca,L} \) was elicited by a voltage pulse (200 ms) to +10 mV from a holding potential of −40 mV. C: averaged peak \( I_{Ca,L} \) density for control (CTL) and LPS myocytes. D: time constant of \( I_{Ca,L} \) decay for both control and LPS myocytes. Data are means ± SE for control and LPS; n = no. of myocytes. *Significantly different from control value under same conditions (P < 0.05).
ing 5 mM Ba\(^{2+}\) and no Ca\(^{2+}\). As shown in Fig. 3, Ba\(^{2+}\) substitution nearly doubled the peak current amplitude for both control and LPS myocytes. However, Ba\(^{2+}\) substitution did not reverse the LPS-induced reduction in peak current. Both peak \(I_{\text{Ca,L}}\) density and peak Ba\(^{2+}\) current (\(I_{\text{Ba}}\)) were elicited by a voltage pulse (200 ms) from a holding potential of \(-40\) mV to +10 mV. C: averaged peak \(I_{\text{Ca,L}}\) and \(I_{\text{Ba}}\) densities for control and LPS myocytes. D: time constant of \(I_{\text{Ca,L}}\) and \(I_{\text{Ba}}\) decay for both control and LPS myocytes. Data are means ± SE for control and LPS myocytes; \(n = \) no. of myocytes. *Significantly different from control value under same conditions (\(P < 0.05\)).

However, there was no difference in the rate of current decay between control and LPS myocytes when Ba\(^{2+}\) was used as the charge carrier (Fig. 3D). Increasing the test pulse duration to 500 ms did not eliminate the difference in the time constant of \(I_{\text{Ca,L}}\) decay between LPS (98 ± 2.2 ms) and control myocytes (80 ± 2 ms, \(P < 0.05\)) when Ca\(^{2+}\) was the charge carrier. Again, the time constants of \(I_{\text{Ba}}\) decay were not different between control (179 ± 11 ms) and LPS (168 ± 22 ms, \(P > 0.05\)) myocytes during a 500-ms test pulse when Ba\(^{2+}\) was used as the charge carrier. These data indicate that the slowed rate of \(I_{\text{Ca,L}}\) decay in LPS myocytes was most likely due to reduced Ca\(^{2+}\) influx.

I-V relationship. To assess the voltage dependence of L-type Ca\(^{2+}\) channels in LPS myocytes, we measured
peak $I_{Ca,L}$ at different voltages using either Ca$^{2+}$ or Ba$^{2+}$ as the charge carrier. Currents of both control and LPS myocytes had similar voltage dependence regardless of whether Ca$^{2+}$ or Ba$^{2+}$ was the charge carrier (Fig. 4). Threshold potential, the potential eliciting maximum peak $I_{Ca,L}$ density, and the reversal potential of $I_{Ca,L}$ were similar between control and LPS myocytes. Despite the similar I-V relationship, averaged peak $I_{Ca,L}$ density of LPS myocytes was significantly decreased at pulse potentials between −10 and +40 mV compared with control values (Fig. 4).

Steady-state $I_{Ca,L}$ activation and inactivation and recovery from inactivation. The voltage dependence of $I_{Ca,L}$ activation was determined as the ratio of peak conductance ($G_{Ca}$) to the maximal peak conductance ($G_{Camax}$) and was expressed as $d_\alpha V = G_{Ca}/G_{Camax}$ and $G_{Ca} = I_{Ca,L}/(V_m - V_{rev})$, where $V_{rev}$ is the apparent reversal potential of $I_{Ca,L}$, and $d_\alpha$ is the steady-state activation parameter (29). When $d_\alpha$ was depicted as the function of test potentials, the activation curve of LPS myocytes shifted slightly toward more positive potentials but was not significantly different from that of control myocytes (Fig. 4C). When the activation curves of individual myocytes were fit to the Boltzmann equation, $d_\alpha = [1 + \exp((V_m - V)/K)]^{-1}$, where $V_m$ is the membrane potential producing half-maximal activation, V is voltage, and K is the slope of the activation curve, neither $V_m$ (1.8 ± 0.3 mV) nor K (5.54 ± 0.8 mV) of LPS myocytes was different from those values of control myocytes ($V_m$: −1.6 ± 0.2 mV; K: 5.52 ± 0.7 mV).

The voltage dependence of steady-state inactivation was determined for both control and LPS myocytes using a double-pulse protocol (10). Figure 5A shows a typical current record obtained with the double-pulse protocol. In this case, a prepulse to −10 mV partially inactivated $I_{Ca,L}$ elicited by a subsequent test pulse to +10 mV. The relative amount of $I_{Ca,L}$ measured at each test pulse was plotted as a function of the prepulse voltage (Fig. 5B). Peak $I_{Ca,L}$ elicited by the test pulse was decreased in both control and LPS myocytes as the prepulse voltage potentials became less negative. Although the absolute values of peak $I_{Ca,L}$ of LPS myocytes were lower than those of control myocytes, the voltage dependence of $I_{Ca,L}$ inactivation was not different from control values (Fig. 5B).

To further confirm that the reduced rate of $I_{Ca,L}$ decay in LPS myocytes was due to reduced Ca$^{2+}$ influx (Figs. 2D and 3D), we measured steady-state inactivation over a broader range of potentials using both Ca$^{2+}$ and Ba$^{2+}$ as the charge carrier. The upturn of $I_{Ca,L}$ inactivation at positive potentials has been taken as evidence of Ca$^{2+}$-dependent $I_{Ca,L}$ inactivation when Ca$^{2+}$ is used as

![Fig. 4. Averaged peak current density-voltage relationships (A and B) and voltage-dependent activation of $I_{Ca,L}$ (C). Peak $I_{Ca,L}$ density-voltage relationship was obtained using Ca$^{2+}$ (A) or Ba$^{2+}$ (B) as the charge carrier. C: for voltage-dependent activation, relative peak conductance ($G_{Ca}/G_{Camax}$) was plotted as a function of test pulse potential (see text). Step changes in voltage were elicited at 6-s intervals. Data points are means ± SE; n = no. of myocytes. *Significantly different from control value under same conditions (P < 0.05).](http://ajpheart.physiology.org.org/ by 10.220.33.5 on April 29, 2017)
A

Fig. 5. Steady-state inactivation of I\(_{\text{Ca,L}}\) obtained from control and LPS myocytes using a double-pulse protocol (see MATERIALS AND METHODS). A: representative current record obtained in response to a 2-pulse protocol with a prepulse of 0 mV (300 ms) and a test pulse of +10 mV (200 ms) separated by a 5-ms resting interval at -40 mV. B: normalized I\(_{\text{Ca,L}}\) (I\(_{\text{Ca,L}}\)/I\(_{\text{Ca,L, max}}\)) of the test pulse plotted as a function of the prepulse potential (see MATERIALS AND METHODS). C: normalized I\(_{\text{Ca,L}}\) of the test pulse plotted as a function of the prepulse potential with either Ca\(^{2+}\) (5 mM) or Ba\(^{2+}\) (5 mM) as the charge carrier. The 2-pulse protocol was slightly modified with a prepulse duration of 150 ms, a test pulse duration of 100 ms, and an interpulse interval of 10 ms (see text). Data are means \(\pm SE; n = \) no. of myocytes.

METHODS). with either Ca\(^{2+}\) or Ba\(^{2+}\), and inactivation was measured with a condition pulse duration of 150 ms and a test pulse duration of 100 ms separated with an interpulse interval of 10 ms. We used higher Ca\(^{2+}\) concentration and slightly modified the two-pulse protocol to ensure the observation of upturn of I\(_{\text{Ca,L}}\) inactivation (16). As shown in Fig. 5C, the inactivation curves reached steady-state level after prepulse voltage of 0 mV and were similar for LPS and control myocytes when Ba\(^{2+}\) was used as the charge carrier. On the other hand, in the presence of 5 mM Ca\(^{2+}\), the maximal degree of inactivation occurred in both control and LPS myocytes at +20 mV of conditioning pulse, at which the peak I\(_{\text{Ca,L}}\) reached maximal. As the conditioning pulse became increasingly more positive, inactivation was relieved (Fig. 5C). Although there is no statistical difference between the inactivation curves of control and LPS myocytes, in the presence of 5 mM Ca\(^{2+}\) the inactivation of control myocytes tended to be larger than that of LPS myocytes at potentials of conditioning pulses where Ca\(^{2+}\) influx was greatest. For example, at +20 mV, inactivation was 78.4 \(\pm 1\)% in control myocytes and 72.9 \(\pm 3\)% in LPS myocytes. These data suggest that the slower rate of Ca\(^{2+}\)-dependent inactivation in LPS myocytes observed in Figs. 2D and 3D was related to the smaller I\(_{\text{Ca,L}}\) of LPS myocytes.

Reduced I\(_{\text{Ca,L}}\) of LPS myocytes could result from a delay in Ca\(^{2+}\) channel recovery from inactivation. We assessed the rate of I\(_{\text{Ca,L}}\) recovery from inactivation using a different double-pulse protocol. A representative tracing is shown in Fig. 6A, demonstrating that Ca\(^{2+}\) current elicited by a test pulse was only partially recovered when the rest interval between prepulse and test pulse was 100 ms. Increasing the rest interval increased the peak current elicited by the test pulses (Fig. 6B). Importantly, the time course of I\(_{\text{Ca,L}}\) recovery from inactivation was similar for control and LPS myocytes.

Effect of Iso on I\(_{\text{Ca,L}}\). L-type Ca\(^{2+}\) channels are modulated by \(\beta\)-adrenergic receptor activation, and channel activity is stimulated through phosphorylation of channel subunits by adenosine 3',5'-cyclic monophosphate (cAMP)-dependent protein kinase (28). The \(\beta\)-adrenergic receptor agonist Iso has been shown to reverse the contractile dysfunction of myocytes isolated from this guinea pig model of endotoxemia (26). Therefore, we evaluated whether Iso also would reverse the reduction in I\(_{\text{Ca,L}}\) of LPS myocytes. In the presence of Iso, I\(_{\text{Ca,L}}\) of both control and LPS myocytes increased compared with I\(_{\text{Ca,L}}\) for the same cell in the absence of Iso (Fig. 7). In this set of myocytes, peak I\(_{\text{Ca,L}}\) density of LPS myocytes (2.7 \(\pm 1.7\) pA/pF) was significantly lower than the value of control myocytes (4.6 \(\pm 0.4\) pA/pF) under basal conditions. In the presence of Iso, peak current density increased 174% in control myocytes and 283% in LPS myocytes (Fig. 7C). I\(_{\text{Ca,L}}\) density was no longer statistically different between these two groups. The enhancement of I\(_{\text{Ca,L}}\) by Iso occurred at pulse potentials between -30 and +40 mV such that in the presence of Iso there was no significant difference in the I-V
relationship between control and LPS myocytes (Fig. 7D). Iso had no effect on threshold potential or the reversal potential but shifted the peak potential for $I_{Ca,L}$ from +10 to 0 mV for both control and LPS myocytes.

Effect of BAY K 8644 on $I_{Ca,L}$. BAY K 8644, a dihydropyridine receptor agonist, has been used widely to stimulate L-type Ca$^{2+}$ channels of various cell types. Enhancement of $I_{Ca,L}$ by BAY K 8644 is independent of cAMP-dependent phosphorylation of Ca$^{2+}$ channels (27, 28). We determined whether BAY K 8644 could overcome the endothoxin-induced reduction in $I_{Ca,L}$. Peak $I_{Ca,L}$ density of LPS myocytes remained significantly less than control values even in the presence of BAY K 8644 (Fig. 8, C and D). Similar to the effect of Iso, BAY K 8644 shifted the I-V relationship such that the pulse potential required for peak current changed from +10 to 0 mV. Neither the threshold potential nor the reversal potential changed in either group of myocytes after exposure to BAY K 8644.

Effect of Iso on systolic [Ca$^{2+}$], transients and cell shortening. To correlate the ability of Iso to reverse endothoxin-induced depression in $I_{Ca,L}$ with myocyte contractile functions, we measured both systolic [Ca$^{2+}$] and myocyte shortening before and after exposure to Iso. Myocytes were loaded with fura 2-AM and field stimulated at 0.5 Hz. Both fura 2 ratios and cell length were recorded simultaneously. LPS myocytes exhibited reduced peak systolic [Ca$^{2+}$], as well as reduced maximal rates of Ca$^{2+}$ rise and fall ($\pm$ dCa$^{2+}$/dt$_{max}$) compared with control myocytes (Table 2). Correlating with reduced systolic [Ca$^{2+}$], cell shortening of LPS myocytes also was decreased (Table 2). Exposure of myocytes to Iso increased peak systolic [Ca$^{2+}$] and cell shortening of both control and LPS myocytes. Iso increased systolic [Ca$^{2+}$] 26% in control and 111% in LPS myocytes over basal values. The relatively greater increase in the size of Ca$^{2+}$ transients of LPS myocytes caused by Iso essentially eliminated the difference in systolic [Ca$^{2+}$], as well as $\pm$ dCa$^{2+}$/dt$_{max}$ between control and LPS myocytes. Furthermore, as predicted by the improved systolic [Ca$^{2+}$], Iso had relatively greater effect on cell shortening of LPS myocytes (26% in control and 156% in LPS) such that there was no significant difference in cell shortening between control and LPS myocytes.

Effect of BAY K 8644 on systolic [Ca$^{2+}$], transients and cell shortening. In contrast to the effects of Iso, BAY K 8644 was ineffective in reversing either contractile dysfunction or reduced systolic [Ca$^{2+}$], of LPS myocytes. Enhancement of systolic [Ca$^{2+}$], and cell shortening by BAY K 8644 was similar between control and LPS myocytes: systolic [Ca$^{2+}$], increased 53% in control and 60% in LPS myocytes (Table 3) and cell shortening increased 50% in control and 66% in LPS myocytes. Thus both systolic [Ca$^{2+}$], and cell shortening of LPS myocytes were still significantly less than respective control values even in the presence of BAY K 8644.

**DISCUSSION**

E. coli endotoxemia consistently produces a deleterious depression in cardiodynamic function, including diminished contractile reserves of left ventricular myocardium and decreased end-diastolic compliance of the left ventricular chamber (23, 32). Our previous work indicated that endothoxin-induced loss of inotropic power can be correlated with reduced systolic [Ca$^{2+}$], and corresponding reduction in cell shortening of individual myocytes (31). In the present study, we determined that myocytes isolated from a guinea pig model of E. coli...
endotoxemia have a shortened action potential duration and decreased peak Ca\(^{2+}\) and Ba\(^{2+}\) currents through L-type Ca\(^{2+}\) channels. Reduction in \(I_{\text{Ca,L}}\) of LPS myocytes could not be attributed to alterations in the \(I-V\) relationship, steady-state inactivation, or recovery from inactivation of the Ca\(^{2+}\) channel. Thus intrinsic voltage-dependent properties of Ca\(^{2+}\) channels appeared normal in LPS myocytes. Activation of Ca\(^{2+}\) channels by the direct Ca\(^{2+}\) channel agonist BAY K 8644 or by the \(\beta\)-adrenergic receptor agonist Iso increased \(I_{\text{Ca,L}}\). However, only Iso reversed the LPS-induced depression in \(I_{\text{Ca,L}}\), systolic [Ca\(^{2+}\)], and cell contraction of ventricular myocytes. These data demonstrate that Ca\(^{2+}\) influx through L-type channels is reduced in ventricular myocytes isolated from LPS-injected animals and that reduced Ca\(^{2+}\) influx may underlie the early myocardial dysfunction associated with gram-negative endotoxemia.

Reduced peak \(I_{\text{Ca,L}}\) density of LPS myocytes is unlikely to be due to experimental conditions associated with myocyte isolation or current recording. We previously established that resting cell length of myocytes from this LPS model is similar to that of control cells and that yields of Ca\(^{2+}\)-tolerant myocytes are similar between control and LPS populations (26). Recording conditions for \(I_{\text{Ca,L}}\) measurement were carefully managed to avoid contamination by other currents and were confirmed by complete current blockade with 0.5 mM Cd\(^{2+}\). Although \(I_{\text{Ca,L}}\) of cardiac myocytes is characterized by time-dependent run down during patch-clamp measurements (4), differences in run down could not account for reduced \(I_{\text{Ca,L}}\) of LPS myocytes. First, the rate of \(I_{\text{Ca,L}}\) run down was similar for the two groups, and second, all recordings were made at the same time after membrane rupture. Thus reduction of \(I_{\text{Ca,L}}\) density in LPS myocytes was not an artifact of recording conditions.

Reduced \(I_{\text{Ca,L}}\) density in LPS myocytes could result from alterations in the voltage-dependent properties of the L-type Ca\(^{2+}\) channel. On the basis of work in dogs,
myocytes isolated from infarcted hearts exhibit a significant reduction in $I_{\text{Ca,L}}$ that is associated with 1) reduced numbers of L-type Ca$^{2+}$ channels, 2) altered voltage-dependent inactivation, and 3) increased rate of $I_{\text{Ca,L}}$ decay (1). However, endotoxemia does not appear to affect the voltage-dependent properties of the L-type Ca$^{2+}$ channels. The $I$-$V$ relationships were similar between control and LPS myocytes when either Ca$^{2+}$ or Ba$^{2+}$ was used as the charge carrier. In addition, Ca$^{2+}$ channels of both control and LPS myocytes exhibited similar voltage-dependent steady-state activation and inactivation, as well as recovery from inactivation. Although the rate of $I_{\text{Ca,L}}$ decay was reduced in LPS myocytes, this reduction appears to be correlated with the reduced peak $I_{\text{Ca,L}}$ in LPS myocytes because substitution with Ba$^{2+}$ as the charge carrier eliminated the difference in the rate of current decay between control and LPS myocytes. These data indicate that endotoxemia does not change the intrinsic voltage-dependent properties of the L-type Ca$^{2+}$ channel.

Elevation in [Ca$^{2+}$]$_i$ decreases the amplitude of $I_{\text{Ca,L}}$ and accelerates $I_{\text{Ca,L}}$ decay through Ca$^{2+}$-dependent inactivation of $I_{\text{Ca,L}}$ in mammalian cardiac myocytes (10, 24, 30). Thus reduced $I_{\text{Ca,L}}$ in LPS myocytes could be explained by elevated [Ca$^{2+}$]$_i$ concentrations in LPS myocytes. In the present study, the pipette solution contained 10 mM EGTA with no added Ca$^{2+}$, which will buffer myoplasmic Ca$^{2+}$ to subnanomolar levels. Thus myoplasmic Ca$^{2+}$ concentration was not different between control and LPS myocytes. More significantly, peak current of LPS myocytes was less than control even when the superfusate Ca$^{2+}$ was replaced with Ba$^{2+}$ as the charge carrier. Ba$^{2+}$ substitution eliminates potential Ca$^{2+}$-dependent inactivation of $I_{\text{Ca,L}}$. which
may persist even in the presence of EGTA (10, 16). Furthermore, the rate of l_{Ca}\, decay in the presence of Ca^{2+} was slower in LPS myocytes compared with control, inconsistent with Ca^{2+}-dependent inactivation of l_{Ca}. In addition, resting [Ca^{2+}] is not different between control and LPS myocytes (31). For example, resting [Ca^{2+}] of fura 2-loaded myocytes used for data in Table 2 (156 ± 11 μM) was similar to that of control myocytes (159 ± 27 μM, P > 0.05) although systolic [Ca^{2+}] was significantly lower in LPS myocytes (Table 2). Thus it is unlikely that [Ca^{2+}]-dependent inactivation is responsible for the reduction of l_{Ca} in LPS myocytes.

Reduced peak l_{Ca} density of myocytes from a cardiac hypertrophy model appears to result from a decrease in sarcolemmal Ca^{2+} channel density possibly due to an increase in myocyte size without concomitant increase in L-type Ca^{2+} channel number (19). However, there is no evidence of myocyte hypertrophy in the endotoxemic guinea pig model used in the current study. Resting length of ventricular myocytes from endotoxemic guinea pigs was not different from that of control myocytes (26), and membrane capacitance was similar for both control and LPS myocytes in the present study. On the other hand, decreases in L-type Ca^{2+} channel numbers independent of myocyte hypertrophy appear to underlie the reduced peak l_{Ca} density in human cardiac myocytes dissociated from failing hearts (20) and myocytes isolated from 5-day infarcted canine hearts (1). In addition, Ca^{2+} channel numbers measured by dihydropyridine binding assays are reduced in cardiac sarcolemmal membranes from endotoxemic rabbits (17). Although present data do not rule out reduced number of membrane channels as causative in the decreased l_{Ca} of LPS myocytes, the ability of ISO to reverse the endotoxin-induced reduction in l_{Ca} density suggests strongly that the absolute number of Ca^{2+} channels of LPS myocytes is similar to that of control cells.

L-type Ca^{2+} channel function can be modulated by β-adrenergic receptor activation (15) and by the direct channel agonist, BAY K 8644 (27, 28). Direct binding of dihydropyridine agonists such as BAY K 8644 to Ca^{2+} channels enhances Ca^{2+} current by increasing the open time and shortening the close time of single channels (28). β-Adrenergic receptor activation also increases channel activity by prolonging the open time and shortening the close time of Ca^{2+} channels. In addition, β-adrenoceptor activation also increases the probability that a channel will open, as reflected by an increase in the number of channel openings per unit time during single-channel recording (12, 28). The β-adrenoceptor-dependent increase in the probability that a channel

### Table 2. Cell shortening and systolic [Ca^{2+}] of control and LPS myocytes before and after treatment with ISO

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>LPS</th>
<th>+dL/dt, μm/s</th>
<th>Control</th>
<th>LPS</th>
<th>−dL/dt, μm/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>11.3 ± 0.8</td>
<td>6.4 ± 0.6</td>
<td>34.8 ± 4.5</td>
<td>21.6 ± 4.1</td>
<td>35.8 ± 3.7</td>
<td></td>
</tr>
<tr>
<td>ISO</td>
<td>14.3 ± 2.0</td>
<td>16.4 ± 1.3</td>
<td>43.2 ± 6.4</td>
<td>46.7 ± 6.0</td>
<td>51.6 ± 8.2</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3. Cell shortening and systolic [Ca^{2+}] of control and LPS myocytes before and after treatment with BAY K 8644

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>BAY K 8644</th>
</tr>
</thead>
<tbody>
<tr>
<td>%CS</td>
<td>12.1 ± 0.9</td>
<td>18.1 ± 0.6</td>
</tr>
<tr>
<td>Basal</td>
<td>8.6 ± 0.8</td>
<td>14.8 ± 0.9</td>
</tr>
<tr>
<td>BAY K 8644</td>
<td>36.7 ± 3.5</td>
<td>55.5 ± 7.5</td>
</tr>
<tr>
<td>+dL/dt, μm/s</td>
<td>30.0 ± 3.6</td>
<td>46.4 ± 4.2</td>
</tr>
<tr>
<td>Control</td>
<td>44.0 ± 4.4</td>
<td>57.1 ± 4.5</td>
</tr>
<tr>
<td>LPS</td>
<td>34.8 ± 2.5</td>
<td>46.3 ± 3.8</td>
</tr>
<tr>
<td>−dL/dt, μm/s</td>
<td>44.0 ± 4.4</td>
<td>57.1 ± 4.5</td>
</tr>
<tr>
<td>Basal</td>
<td>4.66 ± 1.8</td>
<td>22.11 ± 3.5</td>
</tr>
<tr>
<td>BAY K 8644</td>
<td>2.63 ± 0.4</td>
<td>13.68 ± 5.1</td>
</tr>
<tr>
<td>+dCa^{2+}/dL, μM/s</td>
<td>3.49</td>
<td>12.68 ± 3.4</td>
</tr>
<tr>
<td>Control</td>
<td>1.56 ± 0.2</td>
<td>4.26 ± 1.0</td>
</tr>
<tr>
<td>LPS</td>
<td>3.72</td>
<td>5.12</td>
</tr>
</tbody>
</table>

Values are means ± SE of last 6 contractions immediately before (basal) and 3 min after ISO perfusion for control (n = 12) and LPS myocytes (n = 12). Fura 2-loaded myocytes were perfused with normal Krebs-Henseleit solution and stimulated at 0.5 Hz for 3 min (basal) and then superfused with Krebs-Henseleit solution containing 0.1 μM ISO. Cell shortening and free intracellular Ca^{2+} concentration ([Ca^{2+}]) were recorded simultaneously. %CS, percent cell shortening from resting length; +dL/dt, maximal rate of cell shortening; −dL/dt, maximal rate of cell shortening; +dCa^{2+}/dL, maximal rate of Ca^{2+} rise; −dCa^{2+}/dL, maximal rate of Ca^{2+} fall. *Significantly different from control value under same conditions (P < 0.05).
will open appears to be dependent on cAMP-dependent protein kinase A (PKA)-mediated phosphorylation of Ca\(^{2+}\) channel subunits (12, 15, 28). Thus the primary difference between these agents is that β-adrenergic receptor agonists increase the probability that a Ca\(^{2+}\) channel will open (28), although both agonists increase macroscopic Ca\(^{2+}\) current without increasing either the number of Ca\(^{2+}\) channels or the single-channel conductance (28).

In the present study, both Iso and BAY K 8644 increased I_{Ca,L} of control and LPS myocytes. However, only Iso reversed the endotoxin-induced reduction of I_{Ca,L} as reflected by the relatively greater increase in I_{Ca,L} of LPS myocytes compared with controls. In contrast, BAY K 8644 produced similar or parallel increases in I_{Ca,L} in both control and LPS myocytes as evidenced by reduced peak I_{Ca,L} density of LPS myocytes in the presence of BAY K 8644. These data suggest that under basal conditions, LPS myocytes may have fewer Ca\(^{2+}\) channels that are available to open. Whether a channel is available to open appears to depend on the phosphorylated state of the channel protein. Phosphorylation-dephosphorylation controls the cycling of individual cardiac L-type Ca\(^{2+}\) channels between two gating modes (11, 12). In the phosphorylation mode, the channel is believed to be available to open, but in the dephosphorylated mode the channel remains less available or silent (11, 12). Thus increased channel phosphorylation via activation of PKA increases the number of channels available to open without necessarily altering the total number of channels resident in the sarcolemma. Data from the present study suggest that endotoxemia somehow decreases the basal phosphorylation state of the L-type Ca\(^{2+}\) channel of cardiac myocytes, resulting in reduced peak I_{Ca,L} density, depressed systolic [Ca\(^{2+}\)], and impaired contractility of these cells.

The ability of Iso to reverse endotoxin-induced reductions in both peak I_{Ca,L} density and peak systolic [Ca\(^{2+}\)] is an important finding, indicating that cardiac responses to β-adrenergic receptor stimulation are conserved in the guinea pig model during early stages of endotoxemia (26). Endotoxemia and septicemia commonly evoke increased concentrations of circulating catecholamines as part of the sympathetic compensatory attempt to maintain cardiac output and circulation to vital tissues (14). Sympathetic support of cardiac work effort will be of limited duration because prolonged exposure of the myocardium to catecholamines typically leads to desensitization or downregulation of the cardiac β-adrenoceptor population (14, 25). Present studies with Iso indicate clearly that cardiac β-adrenoceptors controlling myocardial inotropy are functionally operative for at least the first 4 h of endotoxemia, but these studies do not address putative loss of such receptors during the later hypotensive and decompensatory stages of this form of endotoxocisis (21, 22). In any case, an endotoxin-induced diminution of the basal phosphorylation state of L-type Ca\(^{2+}\) channels, as we now propose, would modulate a mechanistic pathway used by sympathetic compensatory attempts to provide increased inotropic support of myocardial performance (14).

In conclusion, ventricular myocytes isolated from endotoxemic guinea pigs exhibited reduced peak I_{Ca,L} density that correlated with decreased systolic [Ca\(^{2+}\)], and decreased cell shortening. Reduced peak I_{Ca,L} density, decreased systolic [Ca\(^{2+}\)], and diminished inotropic capability of LPS myocytes were selectively reversed by β-adrenoceptor stimulation with Iso, but not by direct Ca\(^{2+}\) channel activation with the dihydropyridine agonist BAY K 8644. These data indicate that reduced Ca\(^{2+}\) influx through L-type Ca\(^{2+}\) channels plays a central role in myocardial contractile dysfunction during endotoxemia.

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