Effects of dexamethasone and 1-canavanine on the intracellular calcium-contraction relation of the rat tail artery during septic shock

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Septic shock is characterized by systemic hypotension and hyporeactivity to vasoconstrictor agonists. The intracellular mechanism by which sepsis produces vascular hyporeactivity is uncertain. Work in animals (mainly LPS models of endotoxemia) suggests that increased production of nitric oxide (NO) may be a major factor in the development of vascular hyporeactivity. Theoretically, NO production could act through dephosphorylation and activation of myosin light chain (MLC) phosphatase by a cGMP-dependent protein kinase (cGK) and/or a fall in intracellular Ca2+ concentration ([Ca2+]i) by phosphorylation of inositol 1,4,5-trisphosphate receptor-associated cGK substrate (26). The former mechanism could produce vasodilatation by lowering the [Ca2+]i sensitivity of the smooth muscle cell (SMC) contractile apparatus downstream of [Ca2+]i mobilization (30). This has been shown in the mesenteric artery of a murine LPS model (20). However, other mechanisms independent of NO production may contribute to alterations in the [Ca2+]i sensitivity of the SMC (16, 20).

Glucocorticoids and inhibitors of inducible NO synthase (iNOS) have been demonstrated to restore vascular reactivity in experimental models of sepsis (19, 23, 28) as well as in human septic shock (4, 33). The cellular mechanisms by which both drugs improve vasoreactivity are not known. Glucocorticoids might restore vasoreactivity in sepsis only through inhibition of iNOS (24, 35). However, we previously found that their effects on global hemodynamics and overall survival in an animal model of polymicrobial sepsis were different from those of selective inhibitors of iNOS (19). These findings suggest that factors other than NO production may contribute to vascular hyporeactivity.

Using the same rodent model (19), we investigated whether sepsis induced abnormalities in resting [Ca2+]i levels and [Ca2+]i sensitivity of arterial SMC contraction. In addition, we postulated that glucocorticoids and iNOS inhibitors may have different effects on these abnormalities. [Ca2+]i sensitivity of contraction was measured in perfused tail arteries removed 24 h after cecal ligation and puncture (CLP) and loaded with the [Ca2+]i-sensitive dye fura 2 in vitro. Intracellular [Ca2+]i concentration and perfusion pressure were measured simultaneously. The rightward shift of the perfusion pressure-intracellular Ca2+ mobilization curve after norepinephrine stimulation subsequent to CLP indicates decreased intracellular Ca2+ sensitivity of contraction. The relation was restored by dexamethasone (which also restored in vivo blood pressure and flow), but not by 1-canavanine (which restored perfusion pressure by further mobilization of intracellular Ca2+). We conclude that CLP lowers vasomotion by lowering intracellular Ca2+ sensitivity, which can be restored with glucocorticoid treatment. The involvement of inducible NO does not solely account for the sepsis-induced reduction in Ca2+ sensitivity of contraction.

glucocorticoid; nitric oxide; sepsis

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Am J Physiol Heart Circ Physiol 291: H1177–H1182, 2006. First published April 7, 2006; doi:10.1152/ajpheart.00997.2005.—The intracellular mechanism by which sepsis lowers vascular reactivity and the subsequent reversal by dexamethasone or nitric oxide synthase (NOS) inhibitors remain unclear. We measured the sensitivity of contraction of the rat tail artery to intracellular Ca2+ in a model of polymicrobial septic shock. At 22 h after cecal ligation and puncture (CLP), rats were treated with an anti-inflammatory glucocorticoid (dexamethasone, 1 mg/kg ip), an inducible NOS inhibitor (1-canavanine, 100 mg/kg ip), or saline. At 24 h after CLP, endothelin-2 denuded, perfused segments of tail artery were loaded with the intracellular Ca2+-sensitive dye fura 2 in vitro. Intracellular Ca2+ concentration and perfusion pressure were measured simultaneously. The rightward shift of the perfusion pressure-intracellular Ca2+ mobilization curve after norepinephrine stimulation subsequent to CLP indicates decreased intracellular Ca2+ sensitivity of contraction. The relation was restored by dexamethasone (which also restored in vivo blood pressure and flow), but not by 1-canavanine (which restored perfusion pressure by further mobilization of intracellular Ca2+). We conclude that CLP lowers vasomotion by lowering intracellular Ca2+ sensitivity, which can be restored with glucocorticoid treatment. The involvement of inducible NO does not solely account for the sepsis-induced reduction in Ca2+ sensitivity of contraction.

Glucocorticoids and inhibitors of inducible NO synthase (iNOS) have been demonstrated to restore vascular reactivity in experimental models of sepsis (19, 23, 28) as well as in human septic shock (4, 33). The cellular mechanisms by which both drugs improve vasoreactivity are not known. Glucocorticoids might restore vasoreactivity in sepsis only through inhibition of iNOS (24, 35). However, we previously found that their effects on global hemodynamics and overall survival in an animal model of polymicrobial sepsis were different from those of selective inhibitors of iNOS (19). These findings suggest that factors other than NO production may contribute to vascular hyporeactivity.

Using the same rodent model (19), we investigated whether sepsis induced abnormalities in resting [Ca2+]i levels and [Ca2+]i sensitivity of arterial SMC contraction. In addition, we postulated that glucocorticoids and iNOS inhibitors may have different effects on these abnormalities. [Ca2+]i sensitivity of contraction was measured in perfused tail arteries removed 24 h after cecal ligation and puncture (CLP) and loaded with the [Ca2+]i-sensitive dye fura 2-AM (9). Contraction was estimated from increases in perfusion pressure after electromechanical (high extracellular K+ concentration) or pharmacomechanical [norepinephrine (NE)] stimulation (29). A subgroup of animals was treated with the anti-inflammatory glucocorticoid dexamethasone, which restores vasoreactivity in the CLP model (19). Inasmuch as the first part of our experimentation showed a reduction in [Ca2+]i sensitivity of contraction in CLP, the involvement of iNOS in this decrease in SMC [Ca2+]i sensitivity was investigated using 1-α-amino-γ-guanidinoxy-n-butyric acid (1-canavanine), an L-arginine analog that is a wide-range inhibitor of arginine-utilizing enzymatic reactions with a certain selectivity for iNOS (15, 17).

MATERIALS AND METHODS

All procedures were conducted in accordance with French law on animal experimentation (permit no. 03-575), after protocol approval by our Institutional Animal Care and Use Committee.

Animal model. Adult male Wistar rats (Charles River, L’Arbresle, France; 400–480 g body wt) were housed at a constant temperature (22°C) and exposed to a 12:12-h light-dark cycle (lights on 0700–1900) for ≥1 wk before use in the experimental protocols. On the penultimate day, the animals were fasted overnight with free access to water. The animals were randomized to one of seven groups: I) control, i.e., no surgery, 2) sham, i.e., animals subjected to laparotomy with...
manipulation of the cecum but without ligation and perforation, 3) sham-dexamethasone, i.e., sham-operated animals treated with dexamethasone (1 mg/kg ip) 22 h after manipulation of the cecum, 4) sham-t-canavanine, i.e., sham-operated animals treated with t-cana-
vanine (100 mg/kg ip) 22 h after manipulation of the cecum, 5) CLP.

t.i., CLP-induced sepsis, 6) CLP-dexamethasone, i.e., sepsis-exposed animals treated with dexamethasone (1 mg/kg ip) 22 h after CLP, and 7) CLP-t-canavanine, i.e., sepsis-exposed animals treated with t-
canavanine (100 mg/kg ip) 22 h after CLP.

Sepsis was induced by CLP as described by Wichterman et al. (34)
with minor modifications. Briefly, the rats were anesthetized with
ketamine (150 mg/kg ip), and a 3- to 4-cm abdominal incision was
made to expose the cecum. The cecum was ligated and punctured once
with a 21-gauge needle, and a small amount of feces was extruded.
The bowel was returned to the abdomen, and the abdominal cavity
was closed. The sham operation consisted of laparotomy and manip-
ulation of the cecum without ligation or puncture. The animals were
resuscitated with saline (5 ml/100 g body wt sc, 0.15 M NaCl) on
completion of surgery to closely represent first-line treatment of septic
shock in humans. Surgical procedures were performed during the
morning, and the animals were carefully observed for 24 h after
surgery.

Rats of each group were randomly allocated to two subgroups: one
for measurement of hemodynamic parameters and plasma nitrite/
nitrate concentrations and another for ex vivo measurement of vaso-
constriction and [Ca$^{2+}$], mobilization in endothelium-denuded rat tail
artery segments.

**Hemodynamic and nitrite/nitrate measurements.** At 24 h after CLP
or the sham procedure, mean arterial pressure (MAP), heart rate, and
abdominal aortic blood flow (ABF) were recorded, and plasma nitrite/
nitrate concentrations were measured.

The animals were anesthetized with thiopental sodium (60 mg/kg
body wt ip), with additional doses (15 mg/kg ip) administered as
necessary to maintain the absence of interdigital reflexes.

The rats were intubated and mechanically ventilated with room air
(50 strokes/min, 10 ml/kg). Body temperature was monitored with
a rectal probe. The left carotid artery was cannulated, and arterial blood
pressure and heart rate were continuously monitored using a dispos-
able pressure transducer (model 5265014, Viggo-Spectramed,
Bilthoven, The Netherlands) and an amplifier-recorder (Sirecust
302A, Siemens, Berlin, Germany). The supraprenal abdominal aorta
was carefully freed from surrounding tissue, and a perivascular probe
(model 1RB, Transonic Systems, Ithaca, NY) was placed around the
aorta. ABF was measured with a small-animal flowmeter (model

Arterial blood samples were obtained from the left carotid artery
after hemodynamic measurements and deproteinized using sulfosal-
cyllic acid, centrifuged, and added to a buffer containing 5% NH$_4$Cl
and 5% NaOH. The samples were injected into a column filled with
copper-plated cadmium filings to reduce nitrate to nitrite. The column
effluent was mixed with Griess reagent. Nitrite concentration was
determined by measurement of the absorbance at 546 nm and com-
pared with a standard solution of sodium nitrate (13). Normal values
were <4 × 10$^{-5}$ M.

**Vasoconstriction and [Ca$^{2+}$], mobilization measurements.** At 24 h
after surgery, the tail artery was dissected out under anesthesia with
thiopental sodium (60 mg/kg body wt ip), with additional doses (15
mg/kg ip) administered as necessary to maintain the absence of
interdigital reflexes. The technique for measurement of vasoconstric-
tion and [Ca$^{2+}$], mobilization has been previously described (9, 10).

Briefly, cannulated segments of the tail artery were placed in a
spectrofluorometer cuvette (Fluorolog FT111, SPEX, Edison, NJ),
and the preparation (lumen and bath) was continually perfused with a
physiological salt solution (PSS; in mM: 140 NaCl, 5 KCl, 1.5 CaCl$_2$,
1 MgCl$_2$, 6 glucose, and 10 HEPES) in 100% O$_2$ at pH 7.40 ± 0.01
and 37°C at a rate of 1.5 ml/min. The endothelium was removed by
brief coperfusion of air (3). The absence of endothelial cells was
confirmed using the silver nitrate en face staining technique (11) (data
not shown). We previously validated the use of the fura 2-loaded,endothelium-denuded rat tail artery for measurement of [Ca$^{2+}$],
sensitivity of SMC contraction (31).

Baseline perfusion pressure (mmHg) and tissue autofluorescence
(excitation at 300–400 nm, emission at 510 nm) were measured. Fura
2 was loaded by perfusion of PSS containing fura 2-AM (5
mg/ml) for 1 min. Fura 2 loading was confirmed using the silver nitrate en face staining technique (11) (data
not shown). We previously validated the use of the fura 2-loaded,
endothelium-denuded rat tail artery for measurement of [Ca$^{2+}$],
sensitivity of SMC contraction (31).

$$[\text{Ca}^{2+}]_i = K_d \times (R_{340/380} - R_{	ext{min}})/(R'_\text{max} - R_{340/380}) \times \beta$$

Where $R_{340/380}$ is the ratio of fluorescence at 340 nm to fluorescence
at 380 nm, $R_{\text{max}}$ is the fluorescence ratio ($R_{340/380}$) at saturating Ca$^{2+}$
(4 × 10$^{-3}$ M CaCl$_2$) plus the Ca$^{2+}$ ionophore ionomycin (10$^{-5}$ M, 5
min), $R_{\text{min}}$ is the fluorescence ratio in Ca$^{2+}$-free PSS containing 10$^{-2}$
M EGTA and 10$^{-5}$ M ionomycin (7 min), $\beta$ is the ratio of fluores-
cence at 380 nm at 0 Ca$^{2+}$ to fluorescence at saturating Ca$^{2+}$
concentration. $R_{\text{max}}/R_{\text{min}}$ was used as an indication of the extent of
fura 2 loading. After measurement of background fluorescence, the
sample was perfused with 10$^{-3}$ M MnCl$_2$ and 10$^{-5}$ M ionomycin for
5 min. $K_d$ represents the dissociation constant, which is 224 nM in
PSS in the absence of proteins (14).

In a first series of experiments, perfusion pressure-[Ca$^{2+}$], curves
were determined after perfusion with 10$^{-5}$–10$^{-6}$ M NE for 2 min at
each concentration in tail arteries only from sham and CLP rats ($n =
5$ each). In a second series of experiments, arteries were perfused for
2 min with 80 mM KCl and then with 10$^{-5}$–10$^{-6}$ M NE for 2 min at
each concentration.

**Substances.** All chemicals were purchased from Sigma (St. Quentin
Fallavier, France), Calbiochem (San Diego, CA), or Merck (Darm-
stadt, Germany). Dexamethasone and t-canavanine were diluted in
PSS in a final volume of 0.5 ml/kg and administered by the intraperi-
toneal route. PSS (0.5 ml/kg ip) was administered to non-dexametha-
soné- and non-t-canavanine-treated animals.

**Statistical analysis.** Values are means ± SE. The level of signifi-
cance was set at $P < 0.05$. EC$50$, the concentration of NE producing
a 50% contraction of the maximal response, was calculated by using
the four-parameter logistic equation, according to the Hill model: $y =
minimum + (E_{\text{max}} - minimum)/[1 + 10(x - \log(\text{EC}_{50})]$, where
minimum represents return to baseline and $x$ is concentration (Prism
3 algorithm for statistical analysis, GraphPad Software, San Diego,
CA). One- and two-way repeated-measures ANOVA were used as
appropriate followed by Bonferroni’s post hoc test for multiple
comparisons.

**RESULTS**

In vivo hemodynamic parameters and nitrite/nitrate levels.
CLP induced a 25% fall in MAP ($P < 0.05$ vs. sham) and a
55% fall in ABF ($P < 0.05$ vs. sham; Table 1). Dexamethasone
and t-canavanine completely restored MAP, but only dexam-
ethasone restored ABF, in CLP rats. Dexamethasone
increased MAP in sham rats (+12%, $P < 0.05$ vs. sham). Heart
rates were similar in all groups (control = 402 ± 10 beats/
min). CLP more than doubled plasma nitrite/nitrate levels ($P <
0.05$). In CLP rats, dexamethasone and t-canavanine lowered
nitrite/nitrate levels to values not significantly different from
those of sham animals, whereas they had no significant effect
on sham animals.

Ex vivo vasoconstriction and [Ca$^{2+}$], mobilization measure-
ments. Neither CLP nor drug (dexamethasone or t-canavanine)
treatment affected baseline perfusion pressure, [Ca$^{2+}$], or
autofluorescence of the tail artery (Table 2). Fura 2 loading was
Table 1. Effects of dexamethasone and L. canavanine on hemodynamic parameters and plasma nitrite/nitrate in CLP and sham-operated rats

<table>
<thead>
<tr>
<th></th>
<th>MAP, mmHg</th>
<th>ABF, ml/min</th>
<th>Nitrates/nitrites, μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Unreated</td>
<td>114±4</td>
<td>23±2</td>
<td>20±2</td>
</tr>
<tr>
<td>Sham Unreated</td>
<td>115±5</td>
<td>23±1</td>
<td>24±2</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>129±2</td>
<td>24±2</td>
<td>22±3</td>
</tr>
<tr>
<td>L-Canavanine</td>
<td>118±6</td>
<td>22±3</td>
<td>19±4</td>
</tr>
</tbody>
</table>

CLP

<table>
<thead>
<tr>
<th></th>
<th>MAP, mmHg</th>
<th>ABF, ml/min</th>
<th>Nitrates/nitrites, μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>86±4</td>
<td>10±1</td>
<td>56±8</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>112±4</td>
<td>18±2</td>
<td>18±6</td>
</tr>
<tr>
<td>L-Canavanine</td>
<td>111±4</td>
<td>12±3</td>
<td>21±6</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 6). MAP, mean arterial pressure; ABF, aortic blood flow. Dexamethasone (1 mg/kg ip) and L-canavanine (100 mg/kg ip) were administered 22 h after manipulation of the cecum and cecal ligation and puncture (CLP) and 2 h before hemodynamic measurements and blood sampling. *P < 0.05 vs. control-untrated, sham-untrated, sham, sham-dexamethasone, sham-CLP, and CLP-dexamethasone. †P < 0.05 vs. control-untrated, sham-untrated, sham-CLP, dexamethasone, and CLP-1-dexamethasone. ‡P < 0.05 vs. control-untrated, sham-untrated, sham-dexamethasone, sham-L-canavanine, and CLP-1-dexamethasone.

Table 2. Effects of dexamethasone and L-canavanine on baseline perfusion pressure, baseline \([Ca^{2+}]_i\), autofluorescence, and fluorescence in tail arteries from CLP and sham-operated rats

<table>
<thead>
<tr>
<th></th>
<th>PP, mmHg</th>
<th>([Ca^{2+}]_i), mM</th>
<th>(AF_{340}), counts × 10^4</th>
<th>(AF_{380}) counts × 10^4</th>
<th>(R_{max}/R_{min})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Unreated</td>
<td>23.9±1.3</td>
<td>21.7±3.0</td>
<td>3.3±0.4</td>
<td>3.2±0.4</td>
<td>9.9±1.2</td>
</tr>
<tr>
<td>Sham Unreated</td>
<td>21.2±1.5</td>
<td>21.3±3.5</td>
<td>3.4±0.3</td>
<td>3.3±0.3</td>
<td>9.3±0.5</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>21.9±1.2</td>
<td>25.1±6.0</td>
<td>3.9±0.3</td>
<td>3.6±0.3</td>
<td>8.6±0.6</td>
</tr>
<tr>
<td>L-Canavanine</td>
<td>24±1.6</td>
<td>30±10.0</td>
<td>3.4±0.3</td>
<td>2.7±0.3</td>
<td>8.5±0.7</td>
</tr>
<tr>
<td>CLP</td>
<td>22.2±1.2</td>
<td>22±5.5</td>
<td>3.4±0.3</td>
<td>3.1±0.3</td>
<td>8.9±0.6</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>23±0.7</td>
<td>25±3.2</td>
<td>3.3±0.4</td>
<td>3.1±0.3</td>
<td>8.8±0.6</td>
</tr>
<tr>
<td>L-Canavanine</td>
<td>23±1.8</td>
<td>32±9.0</td>
<td>3.6±0.3</td>
<td>2.8±0.3</td>
<td>7.9±0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 6). Dexamethasone and L-canavanine were administered as described in Table 1. PP, baseline perfusion pressure; AF, autofluorescence. \((R_{max}/R_{min})\), ratio of fluorescence at saturating \([Ca^{2+}]\) to fluorescence at saturating \([Ca^{2+}]\); \(AF_{340}\) and \(AF_{380}\), autofluorescence at 340 and 380 nm; \([Ca^{2+}]_i\), intracellular \([Ca^{2+}]\) concentration.

satisfactory (\(R_{max}/R_{min} = 8–10\)). In CLP rats, NE produced a rapid rise in \([Ca^{2+}]_i\), followed by a slow decay in the fura 2 signal and a slowly developing contractile response (Fig. 1). As shown in Fig. 2A, the NE concentration-perfusion pressure relation was altered in CLP animals, with a rightward shift of \(-\log(EC50)\) from 5.82 ± 0.04 (sham) to 5.65 ± 0.04 (CLP) \((P < 0.05)\) and a reduction in maximal perfusion pressure \((P < 0.05)\). The \([Ca^{2+}]_i\)-NE concentration curve was shifted upward in CLP compared with sham animals \((P < 0.05); \) Fig. 2B). The perfusion pressure-\([Ca^{2+}]_i\) relation, which defines \([Ca^{2+}]_i\) sensitivity, in the CLP group was markedly different from that in the sham group, with a rightward shift of the CLP curve \((P < 0.05)\), indicating a reduced \([Ca^{2+}]_i\) sensitivity of the contractile apparatus (Fig. 2C). Similarly, as shown in Fig. 3A, the \([Ca^{2+}]_i\) increase induced by membrane depolarization (KCl) was higher and resulted in a smaller increase in perfusion pressure in CLP than in sham animals \((P < 0.05)\). Dexamethasone and L-canavanine had no significant effect on perfusion pressure, \([Ca^{2+}]_i\) increase, or \([Ca^{2+}]_i\) sensitivity in sham animals on stimulation with KCl or NE (Fig. 3). In CLP animals, dexamethasone completely restored perfusion pressure and partially restored \([Ca^{2+}]_i\) sensitivity with KCl and NE stimulation. In CLP animals, L-canavanine restored perfusion pressure at the expense of increased \([Ca^{2+}]_i\), and did not improve \([Ca^{2+}]_i\) sensitivity with NE or KCl stimulation.

**DISCUSSION**

Our results show no reduction in resting and stimulated \([Ca^{2+}]_i\) values in the CLP rat model of multibacterial sepsis. Under an electromechanical (high extracellular K+ concentration) or a pharmacomechanical (NE) stimulus, the increase in \([Ca^{2+}]_i\) was greater, but the \([Ca^{2+}]_i\) sensitivity of vascular SMC contraction was lowered. These changes were reversed by dexamethasone. L-Canavanine restored NE-induced contraction but did not improve \([Ca^{2+}]_i\) sensitivity of SMC contraction.

Before we discuss the significance of our findings, we must consider methodological artifacts in measurement of \([Ca^{2+}]_i\), with fluorescent dyes (9), especially in tissues such as arteries, which have a high percentage of highly fluorescent extracellular matrix, the physicochemical properties of which can be altered by NO-induced nitration (22). This does not seem to be a factor here, however, inasmuch as tissue autofluorescence and baseline fluorescence after fura 2 loading were unaffected by CLP.

To our knowledge, the \([Ca^{2+}]_i\) mobilization-contraction relation in vascular SMC has not been investigated in CLP models of sepsis, which are clinically more relevant than LPS models. In an acute LPS-treated animal model, increases in \([Ca^{2+}]_i\) were present in mesenteric arteries (20). Except for basal \([Ca^{2+}]_i\), which remained in the normal range, the present findings are consistent with these experiments. Similar depressed \([Ca^{2+}]_i\) sensitivity has been shown in cardiac myofilaments of LPS (30, 37) or CLP (35) animal models. Finally, a reduction in striated muscle fiber \([Ca^{2+}]_i\) sensitivity has also been observed in the diaphragm of endotoxemic rats (8). Thus these patterns appear to be ubiquitous in septic contractile tissues. Conversely, there is no experimental evidence of reduced \([Ca^{2+}]_i\) at rest and during stimulation in these septic tissues, which is also consistent with the present findings.

The mechanisms responsible for the reduced \([Ca^{2+}]_i\), sensitivity of SMC contraction have yet to be determined. One

CALCIUM SENSITIVITY AND SEPTIC SHOCK

H179
possible mechanism could be the sepsis-induced production of NO. The present model provided indirect evidence of increased NO production through nitrite/nitrate accumulation. L-Canavanine and dexamethasone, which are inhibitors of iNOS, reversed arterial hypotension and nitrite/nitrate production. Although NO-induced membrane hyperpolarization through opening of Ca\(^{2+}\)/H\(^{+}\)-activated (2, 6) or ATP-sensitive (21) K\(^{+}\) channels may account for resistance to vasoconstrictors, the observation that KCl-induced SMC contraction was depressed in CLP animals suggests that other mechanisms are involved. Furthermore, in an endothelium-denuded preparation, such NO-induced membrane hyperpolarization is unlikely. In addition, NO has been previously demonstrated to lower Ca\(^{2+}\)/H\(^{+}\) sensitivity of tension in the rat tail artery without affecting resting and stimulated [Ca\(^{2+}\)]\(_{i}\) (12, 31). The dephosphorylation and activation of MLC phosphatase induced by NO through a cGK could account for this observation (26). The evolution of the contraction with time could be explained on the basis of such a phenomenon. In our fura 2-loaded rat tail artery preparation, the fluorescent fura 2 signal is generally of shorter duration in the CLP than in the sham group, the contraction being maintained by cross-bridging when [Ca\(^{2+}\)]\(_{i}\) has fallen (9, 31). Activation of MLC phosphatase by CLP might explain why the increase in perfusion pressure is of shorter duration in the CLP than in the sham group. Finally, contraction in arteries from septic animals treated with L-canavanine or dexamethasone was similar to that in arteries from control animals. However, although these data suggest that vascular hyporeactivity induced by this polymicrobial model of sepsis is linked to NO production, other mechanisms may explain the alterations in [Ca\(^{2+}\)]\(_{i}\) sensitivity. Indeed, in the septic L-canavanine-treated animals, contraction evoked by depolarization with KCl was unchanged, whereas restoration of NE-induced SMC contraction was observed at the expense of a massive increase in [Ca\(^{2+}\)]\(_{i}\) without improvement in [Ca\(^{2+}\)]\(_{i}\) sensitivity. Thus L-canavanine potentiates NE-induced vasoconstrictive effects probably via effects different from those directly involved in defective [Ca\(^{2+}\)]\(_{i}\) sensitivity. Because L-canavanine improved NE-induced, but not KCl-stimulated, [Ca\(^{2+}\)]\(_{i}\) mobilization in CLP animals, it can be hypothesized that it reversed impairment of an NO-dependent, cGMP-induced Ca\(^{2+}\) release from intracellular stores (26). Thus, in this model, NO production may be implicated in inappropriate [Ca\(^{2+}\)]\(_{i}\) mobilization, mainly through inhibition of Ca\(^{2+}\) release from intracellular stores, whereas NO-independent mechanisms contribute to defective [Ca\(^{2+}\)]\(_{i}\)-contraction coupling, whether or not they are superimposed on NO-induced activation of MLC phosphatase.

In dexamethasone-treated septic animals, almost total restoration of [Ca\(^{2+}\)]\(_{i}\) sensitivity was observed with KCl and NE. Although dexamethasone is an inhibitor of iNOS (23), the differences between the effects of L-canavanine and the effects of dexamethasone with respect to [Ca\(^{2+}\)]\(_{i}\) sensitivity of contraction suggest the involvement of other mechanisms. Several processes and sites of action may account for the enhancement of vascular contractility induced by corticosteroids through \(\alpha\)-adrenergic receptors, angiotensin II receptors, and ion channels and pumps (32). Because the impairment in septic SMC contraction was subsequent to mechanisms downstream of [Ca\(^{2+}\)]\(_{i}\) mobilization and because we observed that beneficial effects of L-canavanine on contraction were linked only to an increase in [Ca\(^{2+}\)]\(_{i}\) mobilization, dexamethasone could act through reversal of an NO-independent impairment in [Ca\(^{2+}\)]\(_{i}\)-contraction coupling. Although speculative, one possibility is an effect of dexamethasone on cyclooxygenase metabolites, which have been demonstrated to contribute to vascular hyporeactivity in septic shock (29). Another possibility is that NO-independent free radical-induced contractile protein dysfunction, as evidenced in septic striated muscle (8), could be
reversed by dexamethasone. Additional investigations are needed to further explore these hypotheses.

What is the potential clinical relevance of these findings? In our septic hypokinetic animal model, the mechanism by which L-canavanine and dexamethasone restored in vivo MAP was different, because L-canavanine further increased vascular resistance, whereas dexamethasone improved ABF. The hemodynamic effects of dexamethasone were more consistent with the usually accepted resuscitation end points, i.e., maintaining or improving oxygen transport and restoring adequate perfusion pressure to tissue, which resulted in more prolonged survival in previous experiments (19). In human septic shock, the experience with selective iNOS inhibitors is very limited. The use of N-monomethyl-L-arginine, a nonselective NOS inhibitor, was associated with a reduction in vasopressor needs (33) but an increase in 28-day mortality (18). Conversely, moderate doses of steroids for 5–7 days reduced the time under vasopressors (5, 7) and mortality (1). The fact that most patients enrolled in these studies had predominant peripheral vascular failure reinforces the concept that sepsis-induced production of NO is not the best target for the hemodynamic management of sepsis.

In conclusion, we found that a decrease in [Ca²⁺]ᵢ sensitivity of SMC contraction was the mechanism responsible for vascular hyporeactivity in a polymicrobial sepsis model. The different effects of dexamethasone and L-canavanine on [Ca²⁺]ᵢ sensitivity of SMC contraction suggest that mechanisms in addition to enhanced NO production may explain the abnormal SMC contraction in sepsis.
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