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

Arnaud Mansart, Pierre-Edouard Bollaert, Philippe Giummely, Christine Capdeville-Atkinson, and Jeffrey Atkinson. Effects of dexamethasone and l-canavanine on the intracellular calcium-contraction relation of the rat tail artery during septic shock. 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 Ca\(^{2+}\) 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 (l-canavanine, 100 mg/kg ip), or saline. At 24 h after CLP, endothelium-denuded, perfused segments of tail artery were loaded with the intracellular Ca\(^{2+}\)-sensitive dye fura 2 in vitro. Intracellular Ca\(^{2+}\) concentration and perfusion pressure were measured simultaneously. The rightward shift of the perfusion pressure-intracellular Ca\(^{2+}\) mobilization curve after norepinephrine stimulation subsequent to CLP indicates decreased intracellular Ca\(^{2+}\) sensitivity of contraction. The relation was restored by dexamethasone (which also restored in vivo blood pressure and flow), but not by l-canavanine (which restored perfusion pressure by further mobilization of intracellular Ca\(^{2+}\)). We conclude that CLP lowers vasomotion by lowering intracellular Ca\(^{2+}\) sensitivity, which can be restored with glucocorticoid treatment. The involvement of inducible NOS does not solely account for the sepsis-induced reduction in Ca\(^{2+}\) sensitivity of contraction.

glucocorticoid; nitric oxide; sepsis

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 (28). 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 Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) by phosphorylation of inositol 1,4,5-trisphosphate receptor-associated cGK substrate (26). The former mechanism could produce vasodilatation by lowering the [Ca\(^{2+}\)]\(_i\), sensitivity of the smooth muscle cell (SMC) contractile apparatus downstream of [Ca\(^{2+}\)]\(_i\), 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 [Ca\(^{2+}\)]\(_i\) 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 [Ca\(^{2+}\)]\(_i\), levels and [Ca\(^{2+}\)]\(_i\), sensitivity of arterial SMC contraction. In addition, we postulated that glucocorticoids and iNOS inhibitors may have different effects on these abnormalities. [Ca\(^{2+}\)]\(_i\), sensitivity of contraction was measured in perfused tail arteries removed 24 h after cecal ligation and puncture (CLP) and loaded with the [Ca\(^{2+}\)]\(_i\)-sensitive intracellular dye fura 2-AM (9). Contraction was estimated from increases in perfusion pressure after electromechanical (high extracellular K\(^+\) concentration) or pharmacomechanical [norepinephrine (NE)] stimulation (27). 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 [Ca\(^{2+}\)]\(_i\), sensitivity of contraction in CLP, the involvement of iNOS in this decrease in SMC [Ca\(^{2+}\)]\(_i\), sensitivity was investigated using l-α-amino-γ-guanidinoxy-n-butyric acid (l-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: 1) 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-cana-vanone (100 mg/kg ip) 22 h after manipulation of the cecum, 5) CLP, i.e., 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-cana-vanone, i.e., sepsis-exposed animals treated with t-cana-vanone (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 manipulation 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 vasoconstriction 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 disposable pressure transducer (model 5265014, Viggo-Spectramed, Bilthoven, The Netherlands) and an amplifier-recorder (Sirecust 302A, Siemens, Berlin, Germany). The suprarenal abdominal aorta was carefully freed from surrounding tissue, and a perivascular probe (model IRB, Transonic Systems, Ithaca, NY) was placed around the aorta. ABF was measured with a small-animal flowmeter (model T206, Transonic Systems). Surgery lasted 20 min.

Arterial blood samples were obtained from the left carotid artery after hemodynamic measurements and deproteinized using sulfosalicylic 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 compared with a standard solution of sodium nitrite (13). Normal values were $<$4 x $10^{-3}$ 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 vasoconstriction and [Ca$^{2+}$], mobilization has been previously described (9, 10). Briefly, cannulated segments of the tail artery were placed in a spectrofluorometer cuvette (Fluorolog F1 T11, 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 x $10^{-6}$ M) for 90 min and washout with PSS for 30 min.

Segments were illuminated alternately (1 s) at excitation wavelengths of 340 and 380 nm. The formula of Grynkiewicz et al. (14), as modified by Scanlon et al. (25), was used to calculate [Ca$^{2+}$], (nM)

$$[Ca^{2+}] = K_d \times (R_{340/380} - R_{min})/(R_{max} - R_{340/380}) \times \beta$$

where R$_{340/380}$ is the ratio of fluorescence at 340 nm to fluorescence at 380 nm, R$_{max}$ is the fluorescence ratio (R$_{340/380}$) at saturating Ca$^{2+}$ (4 x $10^{-3}$ M CaCl$_2$) plus the Ca$^{2+}$ ionophore ionomycin (10$^{-5}$ M, 5 min), R$_{min}$ 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 fluorescence at 380 nm at 0 Ca$^{2+}$ to fluorescence at saturating Ca$^{2+}$ concentration. R$_{max}$/R$_{min}$ was used as an indication of the extent of fura 2 loading. After measurement of background fluorescence, the sample was perfused with 10$^{-4}$ M MnCl$_2$ and 10$^{-3}$ 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 (Darmstadt, Germany). Dexamethasone and t-cana-vanone were diluted in PSS in a final volume of 0.5 ml/kg and administered by the intraperitoneal route. PSS (0.5 ml/kg ip) was administered to non-dexamethasone- and non-t-cana-vanone-treated animals.

**Statistical analysis.** Values are means ± SE. The level of significance 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 + (Emax - minimum)/(1 + 10$^{[(x - log(EC_{50})]})$, 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-cana-vanone completely restored MAP, but only dexamethasone 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-cana-vanone 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 measurements.** Neither CLP nor drug (dexamethasone or t-cana-vanone) treatment affected baseline perfusion pressure, [Ca$^{2+}$], or autofluorescence of the tail artery (Table 2). Fura 2 loading was
CALCIUM SENSITIVITY AND SEPTIC SHOCK

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>Nitr/o/nitrate, µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Untreated</td>
<td>114±4</td>
<td>23±2</td>
<td>20±2</td>
</tr>
<tr>
<td>Sham Untreated</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>
<tr>
<td>CLP 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 flow; [Ca2+]i, intracellular calcium concentration; CLP, cecal ligation and puncture; KCl, potassium chloride; NE, norepinephrine.

Table 2. Effects of dexamethasone and l-canavanine on baseline perfusion pressure, baseline [Ca2+]i, autofluorescence, and fluorescence in tail arteries from CLP and sham-operated rats

<table>
<thead>
<tr>
<th>PP, mmHg</th>
<th>[Ca2+]i, nM</th>
<th>AP340, counts × 10⁶</th>
<th>AP380 counts × 10⁶</th>
<th>Rmax/Rmin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Untreated</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>
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<tr>
<td>Sham Untreated</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>
</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>
</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>
</tr>
<tr>
<td>CLP Untreated</td>
<td>22.2±1.2</td>
<td>22±5.5</td>
<td>3.4±0.3</td>
<td>3.1±0.3</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>
</tr>
<tr>
<td>L-Canavanine</td>
<td>23.1±1.8</td>
<td>32±9.0</td>
<td>3.5±0.3</td>
<td>2.8±0.3</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, AP, autolfuorescence, (Rmax/Rmin), ratio of fluorescence at saturating Ca2+ (Rmax) to fluorescence at saturating Ca2+ (Rmin); AP340 and AP380, autolfuorescence at 340 and 380 nm; [Ca2+]i, intracellular Ca2+ concentration.

Discussion

Our results show no reduction in resting and stimulated [Ca2+]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 [Ca2+]i was greater, but the [Ca2+]i sensitivity of vascular SMC contraction was lowered. These changes were reversed by dexamethasone. L-Canavanine restored NE-induced contraction but did not improve [Ca2+]i sensitivity of SMC contraction.

Before we discuss the significance of our findings, we must consider methodological artifacts in measurement of [Ca2+]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 [Ca2+]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 basal [Ca2+]i, and decreases in sensitivity to [Ca2+]i, were observed in small mesenteric arteries (20). Except for basal [Ca2+]i, which remained in the normal range, the present findings are consistent with these experiments. Similar depressed Ca2+ sensitivity has been shown in cardiac myofilaments of LPS (30, 37) or CLP (35) animal models. Finally, a reduction in striated muscle fiber Ca2+ 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 [Ca2+]i at rest and during stimulation in these septic tissues, which is also consistent with the present findings.

The mechanisms responsible for the reduced [Ca2+]i sensitivity of SMC contraction have yet to be determined. One
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\(^{2+}\)-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\(^{2+}\) 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 dysregulation, 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 hypokinetik 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-nomethyl-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 \([\text{Ca}^{2+}]_i\) 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 \([\text{Ca}^{2+}]_i\) sensitivity of SMC contraction suggest that mechanisms in addition to enhanced NO production may explain the abnormal SMC contraction in sepsis.

Fig. 2. Intracellular \([\text{Ca}^{2+}]_i\) concentration and perfusion pressure responses in perfused tail artery segments exposed to 10^{-8}, 3 \times 10^{-8}, 10^{-7}, 3 \times 10^{-7}, 10^{-6}, 3 \times 10^{-6}, 10^{-5}, and 3 \times 10^{-5} M \text{NE}. Values are means ± SE (n = 5). A: concentration-response curves for \text{NE}-induced increase in perfusion pressure. B: concentration-response curves for \text{NE}-induced increase in \([\text{Ca}^{2+}]_i\). C: \([\text{Ca}^{2+}]_i\)-perfusion pressure relation.

Fig. 3. \([\text{Ca}^{2+}]_i\)-perfusion pressure relation in perfused tail artery segments exposed to 80 mM KCl or 10^{-6} and 10^{-5} M \text{NE}. Values are means ± SE (n = 6). A: KCl (80 mM). B: 10^{-6} M \text{NE} (bottom) and 10^{-5} M \text{NE} (top).
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GRANTS

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