Capillary and arteriolar responses to local vasodilators are impaired in a rat model of sepsis

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Tyml, Karel, Jingcheng Yu, and David G. McCormack. Capillary and arteriolar responses to local vasodilators are impaired in a rat model of sepsis. J. Appl. Physiol. 84(3): 837–844, 1998.—Although sepsis is known to affect vascular function, little is known about changes at the capillary level. We hypothesized that sepsis attenuates the “upstream” arteriolar response to vasoactive agents applied locally to capillaries. Sepsis in rats was induced by cecal ligation and perforation. After 24 h, extensor digitorum longus muscle was prepared for intravital microscopy. Phenylephrine (PE, 10 mM) and acetylcholine (ACh, 10 mM) were applied iontophoretically on terminal arterioles and on their downstream daughter capillaries (300 µm from arteriole). There was no significant difference between control and septic rats in baseline arteriolar diameters [8.0 ± 0.6 vs. 9.8 ± 0.8 (SE) µm] or baseline red blood cell velocity (V_{RBC}) in perfused daughter capillaries (255 ± 10 vs. 264 ± 13 µm/s). Application of PE onto arterioles resulted in comparable constrictions (i.e., ∼22% diameter change) and V_{RBC} reductions (∼100%) in control and septic rats. In contrast, arteriolar diameter and V_{RBC} increases after application of ACh were attenuated in sepsis (diameter: from 41 to 14%; V_{RBC}: from 67 to 24%). Application of PE onto the capillary reduced V_{RBC} to the same level (∼100%) in both groups, whereas application of ACh increased V_{RBC} less in septic than in control rats (20 vs. 73%). On the basis of the arteriolar-capillary pair stimulations, sepsis affected V_{RBC} responses to ACh more in the capillary than in the arteriole. When the adenosine analog 5′-N-ethylcarboxamidotriphenosine (0.1 mM) was used instead of ACh, similar effects of sepsis were seen. To test for a possible involvement of inducible NO synthase (iNOS) in sepsis-induced attenuated ACh responses, arterioles and capillaries in septic animals were locally pretreated with the iNOS blocker aminoguanidine (10 mM). In both microvessels, aminoguanidine restored the ACh response to the control level. We conclude that impaired capillary V_{RBC} and arteriolar diameter responses to vasodilators applied to capillaries in septic rat skeletal muscle were due to dysfunction at arteriolar and capillary levels. The study underscores the significant role of iNOS in sepsis-induced alteration of vascular reactivity in vivo.

capillary; skeletal muscle; nitric oxide

SEPSIS IS A GENERALIZED systemic inflammatory response after a focal infectious or noninfectious insult. Sepsis is characterized by a variety of cardiovascular changes, including a decreased systemic vascular resistance, increased arterial lactate, impaired oxygen utilization (6, 31), and maldistribution of microvascular flow (17). It has been speculated that, despite maintained systemic oxygen delivery, maldistribution of flow may lead to the development of microregional areas of hypoxia, parenchymal cell injury, and eventual multiple organ dysfunction (17). Although the effect of sepsis on the microvasculature has received considerable investigative attention, the mechanism of maldistribution of flow is not well understood. On the basis of several models of sepsis (including endotoxin infusion), numerous studies have reported that sepsis causes constriction in larger arterioles (80–140 µm diameter) (1, 2, 10, 19) and dilation in smaller arterioles (7–80 µm diameter) (1, 7, 10, 19). Sepsis also attenuates responses to vasoconstrictive (2, 5, 15, 27, 35) and vasodilative stimuli (1, 2, 20, 35). Because arterioles contribute significantly to distribution of flow (4), altered vascular diameter and reduced vascular responsiveness to vasoactive agents (13, 22) could play a role in the maldistribution of flow.

Recently, we demonstrated in rat skeletal muscle that local application of vasoactive agents onto capillaries can alter the “upstream” diameter of arterioles that feed these capillaries (25). To explain this phenomenon, we proposed that the cells of the capillary wall (i.e., endothelium and/or pericyte) can sense these local agents and communicate their presence to the arteriole to alter its diameter (32). Sepsis has been shown to have a profound effect on endothelial function in a number of ways, including altered biosynthesis of nitric oxide (NO) and endothelial layer permeability (14, 24). Therefore, it is possible that, in terms of the capillary-sensing and communication phenomenon, any effect of sepsis on endothelial/capillary function per se could also contribute to abnormal arteriolar response and maldistribution of flow.

The overall aim of the present study was to explore this possibility. In particular, we wished to test the hypothesis that sepsis attenuates the upstream diameter response after local application of vasoactive agents onto capillaries. To separate the sepsis-induced effect on the communicated response from the direct effect on arterioles, the two particular objectives were to determine responses to direct arteriolar stimulations with these agents and responses to local capillary stimulations with these agents in the microvasculature of septic rat skeletal muscle.

It has been recognized recently that sepsis-induced excess production of NO, via the inducible form of NO synthase (iNOS), could be responsible for attenuation of vascular contractility (15, 19, 31). Because our preliminary data indicated a sepsis-induced deficit in the responsiveness to locally applied acetylcholine (ACh), we wished to explore the role of iNOS in the communicated response. Accordingly, the third objective was to determine the effect of aminoguanidine (AG, a selective blocker of iNOS) (31) on responses to ACh.
applied locally on arterioles and on capillaries in septic rat skeletal muscle.

METHODS

Animal Preparation

Male Sprague-Dawley rats (2–3 mo old) were randomly divided into two groups: control and septic. The septic group was prepared according to a procedure previously described by our laboratory (17). Briefly, rats were anesthetized via a mask using a gas mixture of halothane (1–2%), oxygen (30–40% oxygen), and nitrogen (remainder). The right carotid artery was cannulated (PE-50 tubing) to permit blood pressure measurement, withdrawal of blood samples, and infusion of fluids. A midline laparotomy was performed, then the cecum was ligated just distal to the ileocecal valve. Next, the cecum was ligated just distal to the ileocecal valve. The hole was covered by a plastic coverslip (18 mm) with a hole (3 mm diameter; surrounded by a silicone ring). The hole was filled with degassed paraffin oil to allow micropipette access to the muscle surface and to prevent the surface from drying. The oil did not contact the muscle surface, inasmuch as a tissue fluid layer (~60 µm thick) was formed between the muscle surface and the oil.

The second preparation allowed for visualization at high magnification (×10 eyepiece and ×32/0.50 NA objective). The preparation included tying a silk ligature around the distal tendon of the EDL muscle, cutting the tendon, and lifting the muscle onto a glass slide fixed to the microscope stage. The muscle surface was covered with plastic wrap (Saran Wrap, Dow Chemical) in which a small rectangle (~2 × 4 mm) was cut to allow micropipette access. This rectangle was filled with degassed paraffin oil to prevent the surface from drying. We could not study responses in larger arterioles or venules (e.g., conducted arteriolar response), inasmuch as only short segments of terminal arterioles and postcapillary venules were visible at the EDL muscle surface.

Local Application of Vasoactive Drugs

Experiments were carried out on microvessels that were clearly visible at the muscle surface and that permitted evaluation of microvascular response (see Evaluation of Microvascular Response). Up to seven microvessels (capillaries or arterioles) were stimulated in each muscle. We studied the terminal portions of arterioles that fed capillaries and that were at least 500 µm long and oriented in parallel to muscle fibers. Arterioles were stimulated within 200 µm from the last capillary bifurcation. Capillaries were stimulated 300 µm downstream from the feeding arteriole. Each microvessel was stimulated only once, unless stated otherwise. Drugs were applied locally by iontophoresis or by pressure ejection. Throughout this study, reported concentrations of drugs were the concentrations in the pipette. Actual tissue concentrations were difficult to determine; nevertheless, they were much less (i.e., ~100–1,000 times less) than the pipette concentrations (26).

Iontophoresis. Glass micropipettes (tip <1 µm diameter) were backfilled with one of the following substances: phenylephrine hydrochloride (PE; Sigma Chemical, St. Louis, MO), ACh chloride (Sigma Chemical), 5'-N-ethylcarboxamidoadenosine (NECA, adenosine analog; RBI, Natick, MA), or AG (Sigma Chemical). PE was dissolved in distilled water; NECA was dissolved in Dulbecco's phosphate-buffered solution (pH 7.4; catalog no. 310-4040AJ, Gibco, Burlington, ON, Canada). ACh was dissolved in phosphate-buffered solution (pH 6.8; composition in mM: 138.9 NaCl, 2.25 KCl, 1.75 KH2PO4, 1.4 Na2HPO4) to prevent drug hydrolysis. AG was dissolved in 0.9% saline solution. Micropipettes were mounted on a micro-manipulator and connected to a microiontophoresis current programmer (model 260, World Precision Instruments). A reference electrode was inserted into the gastrocnemius muscle of the same leg of the animal. Drugs were ejected by a +80-nA current for 10 s. Current-mediated ejection started as soon as the micropipette entered the tissue fluid layer above the selected capillary or arteriole. At the end of the 10-s stimulation period, the micropipette was lifted from the fluid layer.

Pressure ejection. Glass pipettes (tip <5 µm) were backfilled with sodium nitroprusside (SNP; Sigma Chemical) dissolved in Dulbecco's phosphate-buffered solution. Each pipette was connected to Picospitzer II (General Valve), which ejected the drug from the pipette over 5–10 ms using pressurized air at 15–30 psi.
Evaluation of Microvascular Response

The microvascular response to locally applied agents on arterioles could be evaluated in terms of diameter changes of the arteriole in terms of red blood cell velocity ($V_{RBC}$) changes in a daughter capillary that is fed directly from this arteriole. Responses to locally applied agents on capillaries could be evaluated in terms of diameter changes of the upstream feeding arteriole or in terms of $V_{RBC}$ changes in the stimulated capillary. The epi-illuminated EDL muscle preparation was not optimal for diameter measurements, since the microscopic image of the blood vessel wall could not be presented with great sharpness. For this reason, in the majority of experiments we chose to use the low-magnification preparation to evaluate the response in terms of $V_{RBC}$. Nevertheless, in several experiments we also used the high-magnification preparation to directly measure the arteriolar diameter response to obtain confirmatory data in conjunction with our $V_{RBC}$ data.

$V_{RBC}$ in capillaries was measured off-line from videotapes using the video flying spot technique (32). In principle, the measurement consists of visual matching of the velocity of a calibrated video cursor to the velocity of a red blood cell moving in the capillary. We determined the control velocity ($V_{RBC\,con}$) as the average $V_{RBC}$ 1–2 min before drug application. Immediately after stimulation the microcirculatory flow was recorded until the flow returned to the control level or at least to ±15% of $V_{RBC\,con}$. Experiments where $V_{RBC}$ did not return to ±15% $V_{RBC\,con}$ were not used. The duration of a response was defined as the time from the onset of the response to the time when $V_{RBC}$ reached $V_{RBC\,con}$ or a stable level within ±15% of $V_{RBC\,con}$. The response velocity ($V_{RBC\,test}$) was defined as the velocity at its greatest change from $V_{RBC\,con}$. Data are expressed as percent change from $V_{RBC\,con}$: $\Delta V_{RBC\,con} (%) = 100% \times (V_{RBC\,test} - V_{RBC\,con})/V_{RBC\,con}$.

Inner luminal arteriolar diameters were measured off-line from the video screen with resolution of about ±1 µm. The diameters were measured for 1–2 min before stimulation ($D_{con}$) and at the maximum change of the arteriolar diameter within the first 3 min after stimulation ($D_{max}$). We measured arteriolar diameters at sites of best visibility along a 200-µm length of the arteriole proximal to the last capillary bifurcation. Reported diameters were from the site of the maximal diameter change. Data are expressed as percent change from $D_{con}$: $\Delta D (%) = 100% \times (D_{test} - D_{con})/D_{con}$.

Experimental Protocol

After rats were prepared for intravital microscopy (blood pressure measurements and blood samples were collected), control and septic rats were randomized into treatment subgroups involving localized application of PE, ACh, NECA, SNP, AG, or AG + ACh.

To address the first objective of the present study (i.e., direct arteriolar response), randomly selected arterioles were stimulated with a vasoconstrictor (PE, 10 mM), an endothelium-dependent vasodilator (ACh, 10 mM), a putative endothelium-independent vasodilator (NECA, 0.1 mM), or an endothelium-independent vasodilator (SNP, 100 mM). Responses to these agents were evaluated in terms of $V_{RBC}$ changes in the stimulated capillary. We also stimulated arteriolar-capillary pairs (i.e., in random order: first, the capillary and then, 10 min later, its feeding arteriole; or first, the arteriole and then, 10 min later, its daughter capillary) with PE (10 mM), ACh (10 mM), or NECA (0.1 mM). Responses were evaluated in terms of $V_{RBC}$ changes in the capillary.

To address the second objective (i.e., response to capillary stimuli), randomly selected capillaries were stimulated with PE (100 mM), ACh (10 mM), or NECA (0.1 mM). Responses to these agents were evaluated in terms of $V_{RBC}$ changes in the stimulated capillary. These microvessels were different from those studied in the first part of the study. The choice of 10 mM AG in the pipette was based on the assumption that the concentration of agents near microvessels was ~100 times less than that in the pipette and on the report that 0.1 mM AG in a tissue bath preparation inhibited iNOS activity (31).

Data Analysis

Values are means ± SE. They were analyzed using Student's t-test (at $P < 0.05$) with Bonferroni's correction, unless stated otherwise. For each group (i.e., control and septic), analyses of variance were performed among the $V_{RBC\,con}$, $D_{con}$, and baseline systemic parameters in different treatment subgroups to ensure that the control and septic rats came from the same populations. In this study, $n$ indicates the number of blood vessels, and $N$ indicates the number of animals. Statistics were calculated using $n$, unless otherwise stated.

RESULTS

Table 1 shows baseline measurements of body weight, arterial blood gases and lactate, and mean arterial pressure obtained from control and septic rats at the beginning of the intravital microscopic study. For technical reasons, not all systemic parameters could be measured in all control and septic rats. Sepsis was associated with a 2.5-fold increase in lactate. Although septic animals exhibited a slightly lower arterial pres-

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<th>Table 1. Baseline parameters in control and septic rats</th>
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<td><strong>Control</strong></td>
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<td>Body wt, g</td>
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Values are means ± SE. Numbers in parentheses indicate number of rats, except for red blood cell velocity ($V_{RBC}$) and diameter, where numbers represent number of analyzed microvessels. Number of rats used for $V_{RBC}$ measurements were 17 and 28; number of rats used for diameter measurements were 9 and 6 in control and septic groups, respectively. NS and S, nonsignificant and significant differences between groups at $P < 0.05$. |
sure than did control animals, they were not hypotensive or hypoxemic. Table 1 shows also the overall control prestimulation V_{RBC} values in capillaries and the overall control prestimulation diameters in arterioles. There were no significant differences in these parameters between the two animal groups. Postmortem examination of the abdominal cavity at the end of each experiment confirmed the presence of a necrotic cecum and purulent peritoneal fluid in septic rats. By contrast, control rats had a normal peritoneal cavity.

Arteriolar Stimuli

In control rats, direct local arteriolar application of PE caused a reduction in capillary V_{RBC} (i.e., flow stoppage for each stimulation), whereas direct arteriolar application of ACh and NECA increased V_{RBC} (Fig. 1). These responses were not limited to one capillary, but rather they occurred simultaneously in all visible capillaries fed by the stimulated arteriole. This indicated that the effect of stimulation was mediated at the arteriole. In general, the onset of these responses occurred within several seconds after the beginning of the stimulus, while the responses lasted ~4–6 min. In the confirmatory experiments involving diameter measurements (Fig. 2), direct application of PE resulted in arteriolar constriction, whereas ACh, NECA, and SNP resulted in arteriolar dilations. In septic rats, application of PE directly to the arteriole caused a reduction in capillary V_{RBC} that was not significantly different from that in control rats (Fig. 1). In contrast, the increases in V_{RBC} measured in capillaries after arteriolar stimulation with ACh and with NECA were reduced. In general, responses lasted ~2.5–6.5 min. Again, responses in septic rats were not limited to one capillary, but rather they occurred in all visible capillaries fed by the stimulated arteriole. The lack of effect of sepsis on PE-induced reduction in capillary V_{RBC} was matched by the lack of effect of sepsis on PE-induced arteriolar constriction (Fig. 2). The arteriolar vasodilations after direct applications of ACh, NECA, and SNP were significantly reduced in septic animals (Fig. 2).

Capillary Stimuli

In control rats, local capillary PE application reduced V_{RBC}, whereas capillary applications of ACh and NECA increased V_{RBC} (Fig. 3). Sepsis had no significant effect on response to PE, whereas it attenuated responses to ACh and to NECA (Fig. 3). Durations of V_{RBC} responses...
in both animal groups ranged from ~3 to 6 min. Responses occurred not only in the stimulated capillary but also in all visible capillaries fed by the same terminal arteriole.

**Arteriolar-Capillary Pairs**

Paired stimulations in control rats showed no difference between response to capillary and arteriolar stimulations with PE, ACh, and NECA (Fig. 4). In contrast, septic rats demonstrated significantly lower responses to capillary ACh and NECA stimuli than to arteriolar ACh and NECA stimuli. Thus sepsis affected responsiveness more in the capillary than in the arteriole.

**Effect of AG**

In control and septic rats, application of 10 mM AG directly on the arteriole had no significant effect on $V_{\text{RBC}}$ in a daughter capillary fed by the arteriole: $\Delta V_{\text{RBC}} = -32 \pm 18\% (n = 8, N = 4)$ and $6 \pm 18\% (n = 12, N = 7)$ for control and septic rats, respectively. Similarly, application of AG on a capillary had no effect on $V_{\text{RBC}}$ in this capillary: $\Delta V_{\text{RBC}} = -16 \pm 19\% (n = 9, N = 4)$ and $13 \pm 20\% (n = 16, N = 7)$ for control and septic rats, respectively. Local pretreatment with AG did not significantly affect the response to arteriolar or capillary ACh in control rats (Figs. 5 and 6). In septic rats, however, pretreatment with AG significantly increased the responses recorded after arteriolar and capillary application of ACh to levels comparable to those of control rats (Figs. 5 and 6). Thus AG restored the sepsis-induced depressed arteriolar and capillary responses to ACh.

**DISCUSSION**

The major findings of the present study were 1) no effect of sepsis on $V_{\text{RBC}}$ reduction in capillaries, and on vasoconstriction, when PE was applied on the smallest arterioles, 2) sepsis-induced attenuation of $V_{\text{RBC}}$ increases and of vasodilation when ACh and NECA were applied on these arterioles, 3) restoration of the attenuated response to ACh with AG pretreatment, and 4) sepsis-induced attenuation of $V_{\text{RBC}}$ responses to ACh and NECA applied to capillaries.

**Model**

In the present study we have used a model of sepsis that has been characterized in our laboratory (17, 22). On the basis of a CLP procedure, this model is associated with a normal systemic blood pressure, unchanged cardiac output, and elevated lactate (17). The model...
In the present study, acute, 2; Figs. 1 and 2). This proportionality confirmed that, control and septic rats were same animals used in experiments of Fig. 1 rather than arteriolar diameter, because increases that were proportional to the arteriolar con-

For the ensuing acute capillary acute diameter change is almost entirely responsible for the ensuing acute capillary hematocrit, behavior of leukocytes and platelets), the other factors (e.g., capillary diameter, blood viscosity, determination not only by arteriolar diameter but also by arteriolar wall in the present epi-illuminated prepara-

Arteriolar Response in Sepsis

The technique of local application of agents on single arterioles by iontophoresis or by pressure ejection has been used by several investigators (11, 25). A single application confines the exposure to the agent to a small segment of the vasculature (11). In the present study the arteriolar response to vasoactive agents in control and septic rats has been assessed mainly in terms of capillary \( V_{RBC} \). We chose to measure \( V_{RBC} \), rather than arteriolar diameter, because 1) it was technically difficult to obtain sharp images of the arteriolar wall in the present epi-illuminated preparation and 2) the \( V_{RBC} \) change caused by the diameter change has been reported to be a more sensitive index of the response: 30% diameter increase caused 60% \( V_{RBC} \) increase (32). Although the “steady-state” \( V_{RBC} \) is determined not only by arteriolar diameter but also by other factors (e.g., capillary diameter, blood viscosity, hematocrit, behavior of leukocytes and platelets), the acute diameter change is almost entirely responsible for the ensuing acute capillary \( V_{RBC} \) change (26). Vaso-

Active agents resulted in capillary \( V_{RBC} \) reductions/ increases that were proportional to the arteriolar con-

strictions/dilations, respectively (i.e., \( \Delta V_{RBC}/\Delta D \) was \( \sim 2; \) Figs. 1 and 2). This proportionality confirmed that, in the present study, acute \( V_{RBC} \) responses were caused mainly by acute diameter responses and that the \( V_{RBC} \) response was a meaningful and sensitive index of the arteriolar response.

We found no significant sepsis-induced change in the baseline diameter of terminal arterioles (Table 1). This appears to contrast with the literature, where an increase in baseline diameter was found during endotox-

emia, apparently because of the continuous production of NO via lipo polysaccharide (LPS)-induced NOS in the arteriolar wall (1, 7). Our finding is consistent with the lack of effect of sepsis on baseline capillary \( V_{RBC} \) (Table 1). There are two possible explanations for the baseline diameter discrepancy. First, it is possible that the septic EDL muscle had significantly dilated arterioles, which, inadvertently, were missed in our sampling process. Because of the requirement that they be clearly visible and located 200 µm from the last capil-

lary bifurcation, the selection process included the most distal segments of terminal arterioles nearest to the muscle surface. If deeper arterioles or more proximal segments (also inaccessible) were dilated, then the conclusions of the present study would be limited to the population of arterioles examined. The second possibility could be that our model of a milder septic stress could have yielded iNOS and NO levels that were not high enough to cause increases in diameter and \( V_{RBC} \). This explanation is consistent with the reports that the effect of endotoxemia on baseline diameter is propor-
tional to the severity of endotoxemia (7, 10). We cannot distinguish between these two possibilities, since nei-
	her measurements of diameters from deeper layers (or from more proximal sites) nor measurements of NO levels near arterioles in endotoxin-infusion models vs. the CLP model are available.

Septic arterioles retained their ability to constrict to PE (Fig. 2). Reduced (2, 5, 15, 27, 35) and unchanged systemic vasoconstrictive responses (28, 36) have been reported. The reason for this is not known, but it could reflect a time-dependent effect of sepsis on the vascula-
ture [i.e., increased hypocontractility deficit with in-
creased severity of sepsis (36)]. We previously reported attenuated PE-induced contractility in conduit vessels in the present model of sepsis (13, 22). Therefore, the normal contractile response to PE in the present study may not only reflect the dependence of the hypocontractility deficit on severity of sepsis, but also it may reflect the dependence on the particular vascular bed and/or the size of the vessels studied (i.e., conduit vessels vs. terminal arterioles).

The sepsis-induced reduction in vasodilative re-

sponse to ACh (Fig. 2) agrees with the reported attenuation of response to ACh in several models of sepsis (1, 30, 36). The continuous production of NO via LPS-

induced NOS during endotoxemia has been proposed to be responsible for the decreased sensitivity to the relaxing effect of ACh (30). In the present study, AG did fully restore the \( V_{RBC} \) response to ACh in septic rats (Fig. 5). This restoration supports the proposal that iNOS/NO is involved in the decreased sensitivity to ACh in endotoxemia. It also demonstrates that the reduced response to ACh in sepsis was not due to the limited arteriolar capability to dilate, since there existed a potent stimulus (i.e., AG + ACh) that elevated \( V_{RBC} \) well above the level of this reduced response. The
mechanism of reduced sensitivity to ACh is not clear. The literature suggests that downregulation and functional inhibition of constitutive NOS (cNOS) could account for this reduction. Downregulation would be possible, since lowered mRNA levels for endothelial NOS were reported in 1) cytokine/LPS-treated cultured human aortic cells (21) and 2) the heart, lung, and aorta of LPS-treated rats (18). Functional inhibition could occur by means of the reported negative-feedback inhibition of cNOS and of endothelium-dependent vasodilation by the continuously produced NO during sepsis (8, 9, 20, 31). Our data would favor the latter possibility, since it would not seem possible that the mRNA and the cNOS enzyme itself could have been reconstituted over the 5- to 7-min period of AG application.

An intriguing finding was that sepsis reduced the arteriolar diameter response to SNP (Fig. 2). This finding is consistent with that of Schneider et al. (30), who found a decreased sensitivity to the relaxing effect of NO (via NO donor 3-morpholinosydnonimine) in arteries exposed to LPS. The reduction (albeit less than reduction of response to ACh; Fig. 2) suggests that sepsis attenuated the endothelium-independent relaxation of arteriolar smooth muscle. Although the mechanism of this attenuation is not clear, it is of interest that after AG pretreatment the capillary V_{RBC} response (and presumably vasodilation) to the endothelium-dependent agonist ACh was restored (Fig. 5).

Capillary Response in Sepsis

We previously showed that local capillary stimuli result in V_{RBC} changes due to upstream arteriolar diameter changes (12, 25, 26, 32) rather than downstream venular changes. The results from the present study (Figs. 1 and 2) confirm that the changes in V_{RBC} after stimulation of the capillary can be fully accounted for by changes in upstream arteriolar diameter. In our previous studies we confirmed that the upstream arteriolar response after capillary stimulation cannot be explained in terms of diffusion of drugs from the capillary to the arteriole (12, 26). To explain the upstream response, we hypothesized agonist-induced signaling and electrotonic spread upstream along the capillary endothelium (32). To this end, direct evidence for an agonist-induced depolarization/hyperpolarization of capillary endothelium (23) and for conduction of depolarization along the capillary (3) has been provided.

The data in the current study (Fig. 4) demonstrate that a part of the reduced vasodilator response to ACh and NECA was due to abnormal sensitivity of the capillary to these agents and/or abnormal upstream spread and was not purely a consequence of abnormal arteriolar dilation. This is consistent with our hypothesis that sepsis attenuates the upstream response after local application of agents onto capillaries and suggests that there may be deficits in agonist-induced sensing and/or in upstream cell-to-cell communication in sepsis. Further studies are required to address the mechanism(s) responsible for these deficits. Nevertheless, our data demonstrating restoration of the V_{RBC} response after AG administration (Fig. 6) suggest that production of NO in the capillary may be involved.

A recent study suggested that endotoxemia induces an abnormal microvascular control of skeletal muscle oxygenation (29). Because the oxygen sensor may be situated downstream from the arteriole (16), the present findings indicate that the focus of this abnormal control could occur at the capillary level, in terms of an oxygen-sensing deficit and/or an upstream communication deficit. Clearly, in addition to the sepsis-induced arteriolar deficit, abnormal capillary sensing and/or communication could also contribute to maldistribution of flow and oxygen supply. The recognition of the potential role of capillaries in the maldistribution of flow in sepsis represents conceptual advancement in understanding this syndrome.

In summary, we have shown that, in the terminal vasculature at the surface of rat skeletal muscle, sepsis affected the capillary blood flow and arteriolar diameter responses to the locally applied vasodilators ACh and NECA but not to the locally applied vasoconstrictor PE. We have demonstrated for the first time in situ that locally applied AG restored the response to ACh. Our study underscores that NO generated in arterioles and capillaries (25) may play a significant role in sepsis-induced alteration of vascular reactivity in vivo.

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