Effects of *Pseudomonas aeruginosa* endotoxin on vasodilation in the intact spinotrapezius muscle

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Suzuki, Hideyuki, Hiroyuki Ikezaki, Rinku Chandiwala, Dennis Hong, and Israel Rubinstein. Effects of *Pseudomonas aeruginosa* endotoxin on vasodilation in the intact spinotrapezius muscle. *J Appl Physiol* 91: 351–356, 2001.—The purpose of this study was to determine whether short-term exposure to clinically relevant concentrations of *Pseudomonas aeruginosa* lipopolysaccharide (LPS) impairs vasoreactivity of resistance arterioles in the intact spinotrapezius muscle microcirculation and, if so, to determine the mechanisms mediating this response. Using intravital microscopy, we found that 60-min suffusion of *P. aeruginosa* LPS (0.03–3.0 μg/ml) on the in situ hamster spinotrapezius muscle elicited an immediate, profound, and prolonged concentration-dependent vasodilation (*P* < 0.05). This response was reversible once suffusion of *P. aeruginosa* LPS was stopped. Pretreatment with Nω-nitro-L-arginine methyl ester (10.0 μM), a nonselective nitric oxide (NO) synthase inhibitor, but not Nω-nitro-L-arginine methyl ester, abrogated *P. aeruginosa* LPS-induced vasodilation and elicited a small, albeit significant, vasoconstriction. Indomethacin had no significant effects on *P. aeruginosa* LPS-induced responses. *P. aeruginosa* LPS had no significant effects on acetylcholine- and nitroglycerin-induced vasodilation in the spinotrapezius muscle. Collectively, these data indicate that short-term exposure to clinically relevant concentrations of *P. aeruginosa* LPS evokes an immediate, potent, prolonged, and reversible NO-dependent, prostaglandin-independent vasodilation in the spinotrapezius muscle. Hence, the purpose of this study was to begin to address this issue by determining whether short-term exposure to clinically relevant concentrations of *P. aeruginosa* LPS impairs vasoreactivity of resistance arterioles in the intact hamster spinotrapezius muscle and, if so, to determine the mechanisms mediating this response.

**METHODS**

**General Methods**

*Preparation of animals.* The intact hamster peripheral microcirculation was used in these studies. This model is used by us and other investigators to elucidate mechanisms underlying the host inflammatory response to injury, such as evoked by *P. aeruginosa* and LPS, in vivo (7, 14–16, 23–25, 29, 33, 36, 37, 39, 42–46). Adult male golden Syrian hamsters (*n* = 40) weighing 132 ± 3 g were anesthetized with pentobarbital sodium (6 mg/100 g body wt ip). A tracheostomy was performed to facilitate ventilation. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
performed to facilitate spontaneous breathing. A femoral vein was cannulated to inject supplemental anesthesia during the experiment (2–4 mg·100 g body wt −1·h −1). A femoral artery was cannulated to monitor mean arterial pressure and heart rate, which did not change significantly during the course of the experiments (102 ± 4 mmHg and 312 ± 3 beats/min at the start and 98 ± 2 mmHg and 316 ± 4 beats/min at the conclusion of the experiments, respectively; \( P > 0.5 \)). Body temperature was monitored during the experiments and maintained constant (37–38°C) using a heating pad.

The right spinotrapezius muscle was prepared for intravitral microvascular observation as previously described in our laboratory and reports in the literature (13, 19, 26, 27, 42). Briefly, a median skin incision was made along the spine and the loose connective tissue beneath the skin was cut away to expose the muscle surface. The animal was placed on its left side, and the lateral side of the right spinotrapezius muscle was carefully pulled out with blunt dissection. The muscle was spread, ventral surface up, over a plastic baseplate, and its ligature was placed in the horizontal position on a silk thread. Care was taken to maintain a physiological length of the muscle during the procedure. An upper plastic chamber was placed above the muscle and contained the suffusate. The chamber was connected via a three-way valve to a reservoir that allowed continuous suffusion of the muscle with warm (37–38°C) bicarbonate buffer [composition (in mM): 131.9 NaCl, 2.95 KCl, 1.48 CaCl₂, 0.76 MgCl₂, and 11.87 NaHCO₃] bubbled continuously with 95% \( \text{N}_2 \)-5% \( \text{CO}_2 \) (pH 7.4). The chamber was connected via a three-way valve to an infusion pump that allowed controlled administration of \( \text{P. aeruginosa} \) LPS and drugs into the suffusate.

**Determination of arteriolar diameter.** The spinotrapezius muscle microcirculation was transilluminated with a fiber-optic light source (Nikon) and viewed through a Nikon micromouse. Muscle microcirculation was transilluminated with a fiber-optic light source (Nikon) and viewed through a Nikon microscope. The right spinotrapezius muscle was carefully pulled out with blunt dissection. The muscle was spread, ventral surface up, over a plastic baseplate, and its ligature was placed in the horizontal position on a silk thread. Care was taken to maintain a physiological length of the muscle during the procedure. An upper plastic chamber was placed above the muscle and contained the suffusate. The chamber was connected via a three-way valve to a reservoir that allowed continuous suffusion of the muscle with warm (37–38°C) bicarbonate buffer [composition (in mM): 131.9 NaCl, 2.95 KCl, 1.48 CaCl₂, 0.76 MgCl₂, and 11.87 NaHCO₃] bubbled continuously with 95% \( \text{N}_2 \)-5% \( \text{CO}_2 \) (pH 7.4). The chamber was connected via a three-way valve to an infusion pump that allowed controlled administration of \( \text{P. aeruginosa} \) LPS and drugs into the suffusate.

**Experimental Protocols**

**Effects of \( \text{P. aeruginosa} \) LPS on arteriolar diameter.** The purpose of these studies was to determine the effects of short-term suffusion of \( \text{P. aeruginosa} \) LPS on arteriolar diameter in the in situ spinotrapezius muscle. After bicarbonate buffer was suffused for 45 min (equilibration period), increasing concentrations of \( \text{P. aeruginosa} \) LPS (0.03, 0.3, and 3.0 \( \mu \)g/ml) were suffused for 60 min each in a random order. Arteriolar diameter was measured before, every minute during the first 15 min of suffusion, and every 5 min for the next 90 min. At least 45 min elapsed between subsequent suffusions of \( \text{P. aeruginosa} \) LPS. Baseline arteriolar diameter was 52 ± 1 \( \mu \)m at the beginning and 51 ± 2 \( \mu \)m at the conclusion of these experiments. In preliminary studies, we determined that repeated suffusions of 0.03, 0.3, and 3.0 \( \mu \)g/ml \( \text{P. aeruginosa} \) LPS were associated with reproducible increases in arteriolar diameter from baseline: 9 ± 1 and 10 ± 2% (0.03 \( \mu \)g/ml), 19 ± 2 and 17 ± 5% (0.3 \( \mu \)g/ml), and 26 ± 2 and 28 ± 1% (3.0 \( \mu \)g/ml). In addition, suffusion of saline (vehicle) for the entire duration of the experiment had no significant effects on arteriolar diameter (1 ± 1 and 1 ± 2% and the start and conclusion of the experiment, respectively). The concentrations of \( \text{P. aeruginosa} \) LPS used in these studies are based on preliminary studies and previous reports in the literature (10, 18, 21, 32, 35, 41).

**Effects of an NO synthase inhibitor on \( \text{P. aeruginosa} \) LPS-induced vasodilation.** On the basis of the results of the experiments outlined above, we next determined whether pharmacologic inhibition of nitric oxide (NO) synthase attenuates \( \text{P. aeruginosa} \) LPS-induced vasodilation in the in situ spinotrapezius muscle. After the equilibration period, \( \text{P. aeruginosa} \) LPS (0.3 \( \mu \)g/ml) was suffused for 60 min. Once arteriolar diameter returned to baseline, \( N^6 \)-nitro-L-arginine methyl ester (L-NAME), an NO inhibitor (30, 34), or \( N^6 \)-nitro-D-arginine methyl ester (D-NAME), its inactive enantio-mer (each, 10.0 \( \mu \)M), was suffused for 30 min before and during repeated suffusions of \( \text{P. aeruginosa} \) LPS (0.3 \( \mu \)g/ml) for 60 min. Arteriolar diameter was determined during each intervention as outlined above. Baseline arteriolar diameter was 51 ± 1 \( \mu \)m at the beginning and 52 ± 1 \( \mu \)m at the conclusion of these experiments. In preliminary studies, we determined that repeated suffusions of L-NAME and D-NAME (each, 10.0 \( \mu \)M) for 90 min had no significant effects on arteriolar diameter (1 ± 2 and 1 ± 1% for L-NAME and 1 ± 1 and 1 ± 1% for D-NAME). The concentrations of L-NAME and D-NAME used in these studies are based on previous studies in our laboratory and reports in the literature (1, 12, 16, 23, 35, 40, 42, 45, 47). The former has been shown to attenuate NO-dependent vasodilation in the in situ peripheral microcirculation of hamsters (23, 40).

**Effects of indomethacin on \( \text{P. aeruginosa} \) LPS-induced vasodilation.** Suzuki et al. (42) showed that vasodilation elicited by suffusion of Escherichia coli LPS on the in situ hamster spinotrapezius muscle is mediated by prostaglandins. Similar observations were reported in other species and vascular beds (20, 47). The purpose of these studies was to determine whether prostaglandins modulate the vasorelaxant effects of \( \text{P. aeruginosa} \) LPS in this microvascular bed. After the equilibration period, indomethacin (10 mg/kg) was administered intravenously over 30 min using an infusion pump followed by suffusion of \( \text{P. aeruginosa} \) LPS (0.3 \( \mu \)g/ml) on the spinotrapezius muscle for 60 min. Arteriolar diameter was determined during each intervention. Baseline arteriolar diameter was 51 ± 2 \( \mu \)m at the beginning and 51 ± 1 \( \mu \)m at the conclusion of these experiments. In preliminary studies, we determined that intravenous infusion of indomethacin (10 mg/kg) had no significant effects on arteriolar diameter and systemic arterial pressure (1 ± 1% before and 1 ± 1% after, and 101 ± 4 mmHg before and 97 ± 3 mmHg after infusion, respectively). The concentration of indomethacin used in these studies is based on previous studies in our laboratory and a report in the literature and has been shown to inhibit cyclooxygenase in hamsters (14, 37, 42).

**Effects of \( \text{P. aeruginosa} \) LPS on acetylcholine- and nitroglycerin-induced vasodilation.** The purpose of these studies was to determine whether \( \text{P. aeruginosa} \) LPS modulates vascular smooth muscle responsiveness to endogenous and exogenous NO in the in situ spinotrapezius muscle (1, 12, 16, 28, 48). After the equilibration period, acetylcholine (10.0 \( \mu \)M), a receptor- and endothelium-dependent vasodilator, or nitroglycerin (10.0 \( \mu \)M), an NO donor that elicits endothelium-independent vasodilation, was suffused for 7 min. Once arteriolar diameter returned to baseline, \( \text{P. aeruginosa} \) LPS
was dissolved in Na₂CO₃ and diluted in saline to the desired concentration. Nitroglycerin was obtained from Nitroglycerin Chemical (St. Louis, MO). Acetylcholine was obtained from Sigma and Amercholin, and acetylcholine were obtained from Sigma.

**RESULTS**

**Effects of LPS on Arteriolar Diameter**

Suffusion of P. aeruginosa LPS for 60 min elicited a significant, immediate, concentration-dependent increase in arteriolar diameter from baseline in the in situ spinotrapezius muscle (Fig. 1; each group, n = 4 animals; P < 0.05). This response was observed within 5 min after the start of suffusion, lasted 10 min after suffusion was stopped, and returned to baseline within 10 min thereafter (Fig. 2; n = 4 animals; P < 0.05).

**Effects of a NO Synthase Inhibitor on LPS-Induced Vasodilation**

Suffusion of L-NAME, but not D-NAME (each, 10.0 μM), on the in situ spinotrapezius muscle abrogated P. aeruginosa LPS (0.3 μg/ml)-induced vasodilation and unmasked a small, albeit significant, and transient vasoconstriction (7 ± 1% decrease from baseline; Fig. 3; each group, n = 4 animals; P < 0.05). Arteriolar diameter returned to baseline within 15 min after suffusion of L-NAME and P. aeruginosa LPS was stopped (Fig. 3).

**Effects of Indomethacin on LPS-Induced Vasodilation**

Pretreatment with indomethacin (10 mg/kg iv) had no significant effects on P. aeruginosa LPS (0.3 μg/ml)-induced vasodilation (Fig. 4; each group, n = 4 animals).

**Effects of P. aeruginosa LPS on Acetylcholine- and Nitroglycerin-induced Vasodilation**

Suffusion of P. aeruginosa LPS (0.3 μg/ml) on the in situ spinotrapezius muscle for 60 min had no significant effects on acetylcholine (10.0 μM) and nitroglycerin (10.0 μM)-induced vasodilation in this microvascular bed (Fig. 5; each group, n = 4 animals).

**DISCUSSION**

There are three new findings of this study. First, we found that short-term suffusion of P. aeruginosa LPS, at concentrations detected in patients with sepsis syndrome (10, 21, 32, 35, 41), on the intact hamster spinotrapezius muscle is associated with an immediate, potent, and prolonged concentration-dependent vasodilation. This response is reversible because arteriolar diameter returned to baseline once suffusion of P. aeruginosa LPS was stopped. The magnitude of the vasodilation was substantial because it corresponds to ~70% reduction in peripheral vascular resistance, a value consistently observed in patients with sepsis syndrome (22, 31, 32, 38). Conceivably, the deleterious effects of P. aeruginosa LPS on vasomotor tone in skeletal muscles could underlie the intractable hypotension observed in patients with P. aeruginosa sepsis syndrome because the largest proportion of resistance...
arterioles that regulate peripheral vascular resistance is present in these muscles (4, 8, 13, 22, 28, 32, 38, 42).

Second, pharmacological inhibition of NO synthase abrogated P. aeruginosa LPS-induced vasodilation and elicited slight, albeit significant, and transient vasoconstriction. Conceivably, inhibition of NO synthase could have shifted the balance of local vasoregulatory mechanisms in the spinotrapezius muscle microcirculation from vasodilation to vasoconstriction. This process was not mediated through local elaboration of vasoconstrictor prostaglandins because indomethacin, at a concentration previously shown to inhibit cyclooxygenase in hamsters (14, 37, 42), had no significant effects on P. aeruginosa LPS-induced responses.

Last, vasodilation elicited by P. aeruginosa LPS was not related to nonspecific effects on the contractile apparatus in intact spinotrapezius muscle microvessels because the magnitude of vasodilation elicited by suffusion of acetylcholine, an endothelium- and NO-dependent vasodilator, and nitroglycerin, an NO donor, on the spinotrapezius muscle was similar before and after suffusion of P. aeruginosa LPS. Collectively, these data suggest that short-term suffusion of clinically relevant concentrations of P. aeruginosa LPS on the intact hamster spinotrapezius muscle resistance arterioles evokes immediate, potent, and prolonged NO-dependent, prostaglandin-independent vasodilation.
Current concepts suggest that elaboration of NO and/or an NO-containing compound(s) is amplified during gram-negative sepsis syndrome predominantly through upregulation of inducible NO synthase (1, 2, 5, 12, 17, 30, 34, 43, 47). The increase in NO output evokes profound, long-lasting vasodilation in the peripheral microcirculation that promotes intractable hypotension (30, 31, 34). Booke et al. (2) showed that S-ethylisothiourea, a non-amino acid inhibitor of NO synthase, reverses vasodilation elicited by live P. aeruginosa in conscious sheep. Inducible NO synthase may have mediated P. aeruginosa LPS-induced vasodilation in the intact hamster spinotrapezius muscle observed in this study. However, the short-term (60 min) duration of exposure coupled with maximal vasodilation observed already within 10 min after the start of P. aeruginosa LPS suffusion do not support this contention. Alternatively, P. aeruginosa LPS may amplify the activity of constitutively expressed isoforms of NO synthase, including inducible NO synthase, in spinotrapezius muscle resistance arterioles by activating tyrosine kinases and/or increasing the availability of enzyme cofactors in target cells (17, 25, 30, 34, 43). Clearly, additional studies using biochemical tools, isoform-selective NO synthase inhibitors, and inducible NO synthase knockout mice are warranted to support or refute this hypothesis.

The observation that exposure to P. aeruginosa LPS elicits immediate, potent, and prolonged vasodilation in the spinotrapezius muscle contrasts with that evoked by E. coli LPS, a bacterium commonly associated with sepsis syndrome in humans (5, 10, 32, 34), in the same preparation (42). Previous work from our laboratory showed that 60-min suffusion of E. coli LPS on the intact hamster spinotrapezius muscle elicits an immediate biphasic vasomotor response consisting of an initial vasoconstriction followed by profound and prolonged vasodilation (42). The former was mediated by angiotensin II, a potent vasoconstrictor peptide, and the latter by reactive oxygen species and vasodilator prostaglandins. Importantly, pharmacological inhibition of NO synthase had no significant effects on E. coli LPS-induced responses. In addition, the deleterious effects of E. coli LPS on vasomotor tone were mediated by proteases released from perivascular mast cells. Taken together, these data suggest that gram-negative bacterial LPS elicits an immediate, profound, and prolonged vasomotor dysfunction in skeletal muscles that is bacterium specific and involves activation of distinct target cells and metabolic pathways in the microcirculation. Although the mechanisms underlying the disparate vasomotor response to P. aeruginosa LPS and E. coli LPS in the intact skeletal muscle microcirculation are uncertain, they may account, in part, for the more severe cardiovascular dysfunction and higher mortality rate observed after exposure to P. aeruginosa LPS than to E. coli LPS in a canine model of septic shock and the relatively poor outcome of patients with P. aeruginosa sepsis syndrome (3, 9, 11, 18, 22). To this end, these data may have important implications for the development of new drugs to treat gram-negative sepsis syndrome by targeting specific metabolic pathways activated by distinct gram-negative pathogens, such as P. aeruginosa and E. coli, in the skeletal muscle microcirculation (31, 34, 42).

In summary, we found that short-term exposure to clinically relevant concentrations of P. aeruginosa LPS evokes an immediate, potent, prolonged, and reversible NO-dependent, prostaglandin-independent vasodilation in the intact hamster spinotrapezius muscle. We suggest this response could play an important role in the pathophysiology of the profound vasomotor dysfunction observed in the peripheral circulation of patients with P. aeruginosa sepsis syndrome.

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