Mast Cell Chymase-Like Protease(s) Modulates Escherichia coli Lipopolysaccharide-Induced Vasomotor Dysfunction in Skeletal Muscle in Vivo

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ABSTRACT

This study investigated whether short-term exposure to Escherichia coli lipopolysaccharide (LPS) elicits vasomotor dysfunction in skeletal muscle in vivo and, if so, whether perivascular mast cell proteases partly modulate this response. With intravital microscopy, we found that suffusion of E. coli LPS on the in situ hamster spinotrapezius muscle for 60 min elicits immediate vasoconstriction followed by vasodilation. Vasoconstriction is abrogated by SK&F 108566, a selective, nonpeptide angiotensin II (AT II) subtype 1 receptor antagonist, chymostatin and soybean trypsin inhibitor. These compounds also attenuate E. coli LPS-induced vasodilation. By contrast, superoxide dismutase, catalase and indomethacin attenuate only E. coli LPS-induced vasodilation. Endothelin receptor antagonists, lisinopril, leupeptin, Bestatin and DL-2-mercaptomethyl-3-guanidinoethylthiopropanoic acid are ineffective. Histochemical analysis of the spinotrapezius muscle reveals abundant perivascular mast cells with chymostatin-inhibitable chymase-like activity. Pretreatment of hamsters with compound 48/80 for 4 days curtails E. coli LPS-induced vasoconstriction and converts vasodilation to vasoconstriction. On balance, these data indicate that E. coli LPS stimulates perivascular mast cells in the in situ hamster spinotrapezius muscle to release an AT II-producing chymase-like protease(s). AT II thus produced elicits local vasoconstriction and elaborates reactive oxygen species which, in turn, generate vasodilator prostaglandins.

Despite recent advances in medical care, Gram-negative bacterial sepsis syndrome remains a major cause of morbidity and mortality among hospitalized patients (Centers for Disease Control and Prevention, 1990; Natanson, 1994). A characteristic feature of this syndrome is vasomotor dysfunction consisting of profound peripheral vasodilation, refractory hypotension and end-organ failure that evolves several hours after exposure to the offending pathogen(s) (Hess et al., 1981; Natanson, 1994; Wurster et al., 1994). The emergence of this triad is considered an ominous prognostic sign associated with high mortality rate (Natanson, 1994). However, the mechanisms underlying the evolution of vasomotor dysfunction in Gram-negative sepsis syndrome are uncertain.

Current concepts suggest that LPS, a macromolecular glycolipid component of Gram-negative bacterial walls, plays an important role in the genesis of vasomotor dysfunction in sepsis syndrome (Natanson, 1994; Neviere et al., 1996; Shenep and Morgan, 1984; Shenep et al., 1988). To this end, skeletal muscle contains a large proportion of resistance arterioles in the peripheral circulation and contributes appreciably to regulation of peripheral vascular resistance under pathophysiological conditions, such as sepsis syndrome (Baker et al., 1992; Cryer et al., 1987; Neviere et al., 1996; Roswell, 1986).

It is well established that resistance arterioles in skeletal muscle and other organs are surrounded by mast cells that release potent phlogistic proteases, including chymase and tryptase, upon stimulation (Gao et al., 1993; Huntley et al., 1985; Li et al., 1993; Raud, 1989; Rubinstein et al., 1990; Shepherd and Duling, 1996; Urbaschek and Urbaschek, 1979). However, the role these proteases play in the pathophysiology of vasomotor dysfunction observed in sepsis syndrome is uncertain (Svensjö et al., 1990; Urbaschek and Urbaschek, 1979). Hence, the purpose of this study was to begin addressing this issue by determining whether short-term exposure to Escherichia coli LPS elicits vasomotor dysfunction in skeletal muscle in vivo and, if so, whether

ABBREVIATIONS: LPS, lipopolysaccharide; AT II, angiotensin II; AT, RA, angiotensin II subtype 1 receptor antagonist; SOD, superoxide dismutase; ACE, angiotensin I-converting enzyme; ET, endothelin; NASDCA, naphthol AS-D chloroacetate
perivascular mast cell proteases partly modulate this response.

Materials and Methods

General Methods

Preparation of animals. Adult male golden Syrian hamsters (n = 80) weighing 137 ± 4 g were anesthetized with pentobarbital sodium (6 mg/100 g b.wt., i.p.). A tracheostomy was performed to facilitate spontaneous breathing. A femoral vein was cannulated to inject supplemental anesthesia during the experiment (2–4 mg/100 g b.wt./h). A femoral artery was cannulated to monitor systemic arterial pressure and heart rate. Both did not change significantly during the course of the experiments. Body temperature was monitored during the experiments and maintained constant (37–38°C) using a feedback controller and heating pad.

The right spinotrapezius muscle was prepared for intravital microscopic observation as described previously (Gray, 1973; Lash and Bohlen, 1987). 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 edge fixed in the horizontal position using 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: NaCl, 131.9; KCl, 2.95; CaCl2, 1.48; MgCl2, 0.76; NaHCO3, 11.87) bubbled continuously with 95% N2-5% CO2 (pH 7.4). The chamber was also connected via a three-way valve to an infusion pump that allowed controlled administration of E. coli LPS and drugs into the suffusate.

Determination of arteriolar diameter. The spinotrapezius muscle microcirculation was transilluminated with a fiberoptic light guide and into a closed-circuit television system consisting of camera (Panasonic WV-1500), monitor (Panasonic TR-124 MA) and videotape recorder (Panasonic AG-1230). The luminal diameter of second-order arterioles in the spinotrapezius muscle (base-line diameter, 51 ± 2 μm) (Fronek and Zwiefach, 1975) was measured from the video display of the microscope image by a videomicrometer (VIA 100; Boeckeler Instruments, Tucson, AZ) as described previously in our laboratory (Gao et al., 1994, 1995; Mayhan and Rubinstein, 1995; Rubinstein et al., 1991). This system was calibrated against a precision line-width standard. In each animal, the same arteriolar segment was used to measure changes in diameter during the experiment.

Experimental Protocols

Effects of E. coli LPS on arteriolar diameter. These studies determined the effects of short-term suffusion of E. coli LPS on the spinotrapezius muscle on arteriolar diameter. After suffusing the bicarbonate buffer for 45 min (equilibration period), increasing concentrations of E. coli LPS (0.3, 3.0 and 30.0 μg/ml) were suffused in random order. Each concentration was suffused for 60 min. 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 E. coli LPS. In preliminary studies, we determined that repeated suffusions of E. coli LPS were associated with reproducible results. The concentrations of E. coli LPS used in these studies are similar to those used previously in the in situ hamster cheek pouch (Gao et al., 1994, 1995; Svensjo et al., 1990).

Mechanisms of E. coli LPS-induced changes in arteriolar diameter. Role of angiotensin II. These studies determined whether local production of AT II partly mediates E. coli LPS-induced vasoconstriction in the spinotrapezius muscle (Baker et al., 1992; Dunn and Horton, 1993). After suffusing the bicarbonate buffer for 45 min, SK&F 108566 (0.1 μM), a selective, nonpeptide AT1, RA (Edwards et al., 1991), was suffused on the spinotrapezius muscle 30 min before and during suffusion of E. coli LPS (3.0 μg/ml) for 60 min. In another series of experiments, SOD (60 U/ml) and SK&F 108566 (0.1 μM) were suffused together 30 min before and during suffusion of E. coli LPS (3.0 μg/ml) for 60 min. Last, AT II (0.05 μM) was suffused for 10 min before and after suffusing SK&F 108566 (0.1 μM) for 30 min. Arteriolar diameter was determined during each intervention.

In preliminary studies, we determined that suffusion of SK&F 108566 (0.1 μM), alone and together with SOD (60 U/ml) for 90 min, was not associated with significant changes in arteriolar diameter. Likewise, suffusion of Na2CO3, the vehicle of SK&F 108566 alone for 90 min had no significant effects on arteriolar diameter. We also found that repeated suffusions of AT II (0.05 μM) for 10 min before and after suffusing saline (vehicle) for 30 min were associated with a reproducible decrease (~20%) in arteriolar diameter from baseline value. The concentration of SOD and AT II used in these studies were previously used in the in situ hamster cheek pouch (Cornish et al., 1979; Del Maestro et al., 1981; Edwards et al., 1991; Erlanson et al., 1990).

Role of reactive oxygen species. These studies determined whether superoxide and hydrogen peroxide, which are elaborated by AT I (McKechnie et al., 1986), partly mediate E. coli LPS-induced responses. After the equilibration period, SOD (60 U/ml) or catalase (60 U/ml) were suffused on the spinotrapezius muscle for 60 min. In another series of experiments, SOD (60 U/ml) or catalase alone for 90 min had no significant effects on arteriolar diameter. The concentrations of SOD and catalase used in these studies are similar to those previously used in the in situ hamster cheek pouch (Del Maestro et al., 1981; Edwards et al., 1991; Erlanson et al., 1990).

Role of endothelin. These studies determined whether ET, which activates the vascular renin-angiotensin system (Rakugi et al., 1990), mediates E. coli LPS-induced responses. After the equilibration period, PD 142893 (1.0 μM), an ET1 receptor antagonist (Mayhan and Rubinstein, 1995), or BQ-485 (1.0 μM), an ET2 receptor antagonist (Itoh et al., 1993), was suffused for 30 min before and during suffusion of E. coli LPS (3.0 μg/ml) for 60 min. Arteriolar diameter was determined during each intervention. In preliminary studies, we determined that suffusion of PD 142893 and BQ-485 alone for 90 min had no significant effects on arteriolar diameter. The concentrations of PD 142893 and BQ-485 used in these studies previously abrogated ET-induced vasoconstriction in hamsters and dogs, respectively (Itoh et al., 1993; Mayhan and Rubinstein, 1995).

Role of prostaglandins. These studies determined whether prostaglandins, which are thought to play a role in the pathophysiology of vasomotor dysfunction in sepsis syndrome (Fujimura and Ebihara, 1990; Natanson, 1994; Warren et al., 1991), mediate E. coli LPS-induced responses. After the equilibration period, indomethacin (10 mg/kg) was administered i.v. for 30 min by an infusion pump followed by suffusion of E. coli LPS (3.0 μg/ml) on the spinotrapezius muscle for 60 min. Arteriolar diameter was determined during each intervention. In preliminary studies, we determined that suffusion of PD 142893, BQ-485 and dimethyl sulfoxide (1.0 μM), the vehicle of PD 142893 and BQ-485, alone for 90 min had no significant effects on arteriolar diameter. The concentrations of PD 142893 and BQ-485 used in these studies previously inhibited cyclooxygenase in the hamster cheek pouch (Gao et al., 1993, 1995; Raud, 1989; Rubinstein et al., 1991).

Role of mast cells and chymase-like proteases. These studies determined whether perivascular mast cells and chymase-like proteases, which convert angiotensin I to AT II in the peripheral circu-
loration (Cornish et al., 1979; Okamura et al., 1990; Reilly et al., 1982; Wintroub et al., 1984), modulate E. coli LPS-induced immediate biphasic vasomotor dysfunction in the in situ spinotrapezius muscle. To accomplish this goal, we adopted four strategies. First, we determined the presence of perivascular mast cells and chymase-like activity in the spinotrapezius muscle and cheek pouch. The latter tissue was used as a positive control (Pearce et al., 1985; Raud, 1989; Shepherd and Duling, 1996; Takai et al., 1996). Hamsters were killed with an overdose of pentobarbital (50 mg/100 g b.wt. i.p.) and placed in Mota's lead acetate solution (1% lead acetate, 50% ethanol and 0.5% acetic acid) for 24 h (Martin et al., 1992). Tissues were then dehydrated in graded solutions of ethanol, embedded in paraffin, cut into 5-μm sections and placed onto glass slides. Tissue sections were deparaffinized in xylene and rehydrated in graded ethanol solutions. To identify mast cells, tissue sections were immersed in toluidine blue solution (0.5% in 0.5 N HCl) for 5 min, rinsed in water, air-dried and coverslipped.

To identify cells containing chymase-like activity, tissue sections adjacent to or near those stained with toluidine blue were subjected to enzyme histochemical staining with NASDCA with minor modifications as described previously (Martin et al., 1992). In this staining reaction, active chymase-like enzyme within mast cells precipitates the azo dye Fast Garnet GBC at the site of production. In toluidine blue-stained sections, extravascular cells containing metachromatic (purple) cytoplasm or granules were considered to be mast cells. In sections stained with NASDCA-Fast Garnet GBC, extravascular cells containing reddish-brown cytoplasm or granules were considered to contain chymase-like activity. To compare the total number of mast cells (represented by metachromatic cells) with the number of cells exhibiting chymase-like (NASDCA-positive) activity, the number of cells in each category were counted and compared in identical regions of adjacent tissue sections. All mast cells in each section were counted using objectives of 10× to 40×.

In a second group of animals, the right spinotrapezius muscle was prepared for intravitral microscopy experiments as outlined above. After the equilibration period, chymostatin (10 μg/ml) or soybean trypsin inhibitor (100 μg/ml), two relatively selective and potent inhibitors of mast cell chymase (Martin et al., 1992; Reilly et al., 1982), was suffused for 30 min before and during suffusion of E. coli LPS (3.0 μg/ml) for 60 min. Arteriolar diameter was determined during each intervention. In preliminary studies, we determined that suffusion of chymostatin (10 μg/ml) and soybean trypsin inhibitor (100 μg/ml) alone for 90 min had no significant effects on arteriolar diameter. The concentrations of chymostatin and soybean trypsin inhibitor used in these studies previously inhibited mast cell chymase (Martin et al., 1992; Okamura et al., 1990; Reilly et al., 1982; Rubinstein et al., 1990). A third group of animals was treated with compound 48/80 (1.5 mg/kg diluted in 0.2 ml saline, once daily i.p.) to deplete mast cells from prerformed mediators (Gao et al., 1993; Raud, 1989; Urschbach and Urschbach, 1979) or saline (0.2 ml) for 4 days. Thereafter, the right spinotrapezius muscle was prepared for intravitral microscopy experiments. After the equilibration period, E. coli LPS (3.0 μg/ml) was suffused for 60 min. Arteriolar diameter was determined during each intervention. The concentration of compound 48/80 used in these studies previously depleted mast cells from preformed mediators in the hamster cheek pouch (Gao et al., 1993; Raud, 1989).

In a fourth group of animals, we determined whether exogenous AT II elicits vasoconstriction by releasing chymase-like protease(s) in the spinotrapezius muscle. After the equilibration period, AT II (0.05 μM) was suffused on the spinotrapezius muscle for 10 min. Once suffusion of AT II was stopped and arteriolar diameter returned to baseline, a mixture of chymostatin (10 μg/ml) and soybean trypsin inhibitor (100 μg/ml) was suffused 30 min before and during suffusion of AT II (0.05 μM) for 10 min. In another group of animals, AT II (0.05 μM) was suffused for 10 min before and after suffusing SK&F 108566 (0.1 μM) for 30 min. Arteriolar diameter was determined during each intervention. The concentration of AT II used in these studies previously elicited potent vasoconstriction in the in situ hamster cheek pouch (Cornish et al., 1979).

Role of other proteases. These studies determined whether proteases other than chymase modulate E. coli LPS-induced responses (Gao et al., 1994; Okamura et al., 1990). In one group of animals, after the equilibration period, lisinopril (10 μM), an ACE inhibitor, was suffused for 30 min before and during suffusion of E. coli LPS (3.0 μg/ml) for 60 min. In a second series of experiments, a mixture of protease inhibitors consisting of leupentin, Bestatin and DL-2-mercaptomethyl-3- guanidinoethylthiopropionic acid (each, 10 μM), to inhibit aminopeptidases, thiol proteinases and carboxypeptidases N, respectively, was suffused for 30 min before and during suffusion of E. coli LPS (3.0 μg/ml) for 60 min. In preliminary studies, we determined that suffusion of lisinopril and the mixture of proteinase inhibitors alone for 90 min had no significant effects on arteriolar diameter. The concentration of lisinopril used in these studies previously inhibited ACE in the hamster cheek pouch (Rubinstein et al., 1995). The mixture of proteinase inhibitors used in these studies was used previously in the in situ hamster cheek pouch (Gao et al., 1994).

Data and Statistical Analyses

When an agent was suffused on the spinotrapezius muscle, we determined the maximal steady-state change in arteriolar diameter and used this as the response to that agent. Arteriolar diameter was expressed as a percentage of the diameter during the control period. Data were expressed as mean ± S.E.M. except for body weight and arteriolar diameter, which were expressed as mean ± S.D. because they characterize the entire sample group and are not compared with another group. Differences between variables were assessed by two-way analysis of variance and the Newman-Keuls multiple range test. A P value of < .05 was considered statistically significant.

Drugs and Reagents

E. coli LPS (serotype 0111:B4), SOD catalase, indomethacin, chymostatin, soybean trypsin inhibitor, AT II, naphtol AS-D chloroacetate and Fast Garnet GBC were obtained from Sigma Chemical Co. (St. Louis, MO). Leupentin and Bestatin were obtained from Peninsula Laboratories (Belmont, CA). DL-2-Mercaptomethyl-3-guanidinioethylthiopropionic acid was obtained from Calbiochem (San Diego, CA). Lisinopril was a gift from Merck & Co. Research Laboratory (Rahway, NJ). SK&F 108566 was a gift from SmithKline Beecham Pharmaceuticals (King of Prussia, PA). BQ-485 was a gift from Banyu Pharmaceutical Co. (Tsukuba, Japan). PD 142893 was a gift from Parke-Davis Pharmaceutical Research (Ann Arbor, MI). All other chemicals were of the highest analytical grade available. SK&F 108566 and indomethacin were dissolved in Na2CO3 and diluted in saline to the desired concentrations on the day of the experiment. BQ-485 and PD 142893 were dissolved in dimethyl sulfoxide and diluted in saline to the desired concentrations on the day of the experiment. All other drugs were dissolved in saline on the day of the experiment.

Results

Effects of E. coli LPS on Arteriolar Diameter

Suffusion of E. coli LPS onto the spinotrapezius muscle for 60 min elicited a significant, concentration-dependent, immediate biphasic vasomotor response consisting of vasoconstriction followed by vasodilation (figs. 1 and 2; P < .05). Maximal vasoconstriction was observed within 4 min of the start of suffusion and maximal vasodilation within 45 min (fig. 1). Arteriolar diameter returned to baseline within 20 min after suffusion of E. coli LPS was stopped. Suffusion of 3.0 μg/ml E. coli LPS elicited a 10.7 ± 0.4% decrease in arteriolar diameter from baseline at 4 min and a 15.8 ± 1.1% increase during each intervention. The concentration of AT II used in these studies previously elicited potent vasoconstriction in the in situ hamster cheek pouch (Cornish et al., 1979).
in arteriolar diameter from baseline at 45 min (fig. 1; n = 37; P < .05 in comparison with baseline). A similar immediate biphasic vasomotor response was observed in larger (A1) and smaller (A3) arterioles of the spinotrapezius muscle (data not shown). Based on these data, we used arteriolar diameter at 4 and 45 min after the start of suffusion of *E. coli* LPS (3.0 μg/ml) in all subsequent data analysis. Suffusion of saline (vehicle) for the entire duration of the experiment was not associated with significant changes in arteriolar diameter from baseline (fig. 1; P > .5).

**Mechanisms of *E. coli* LPS-Induced Changes in Arteriolar Diameter**

**Role of angiotensin II.** SK&F 108566 (0.1 μM) abrogated *E. coli* LPS (3.0 μg/ml)-induced immediate biphasic vasomotor response (fig. 3A; each group, n = 7; P < .05). Arteriolar diameter increased by 4.8 ± 0.8% from baseline at 4 min and by 2.9 ± 1.8% from baseline at 45 min during suffusion of SK&F 108566 (0.1 μM) and *E. coli* LPS (3.0 μg/ml). Suffusion of SK&F 108566 (0.1 μM) together with SOD (60 U/ml) had similar effects on *E. coli* LPS (3 μg/ml)-induced responses (fig. 3B; each group, n = 5; P < .05). SK&F 108566 (0.1 μM) also abrogated AT II (0.05 μM)-induced vasoconstriction (n = 4; P < .05). Arteriolar diameter decreased by 22.7 ± 1.9% from baseline during suffusion of AT II (0.05 μM) alone and by 1.0 ± 1.0% from baseline during suffusion of SK&F 108566 (0.1 μM) and AT II (0.05 μM).

**Role of superoxide and hydrogen peroxide.** SOD (60 U/ml) had no significant effects on *E. coli* LPS (3.0 μg/ml)-induced vasoconstriction (fig. 4A; P > .5). However, it reverted *E. coli* LPS-induced vasodilation to significant vasoconstriction (fig. 4A; n = 7; P < .05). Arteriolar diameter decreased by 12.3 ± 2.6% from baseline at 45 min during suffusion of SOD (60 U/ml) and *E. coli* LPS (3.0 μg/ml). Catalase (60 U/ml) had no significant effects on *E. coli* LPS (3.0 μg/ml)-induced vasoconstriction (fig. 3B; P > .5). However, it significantly attenuated *E. coli* LPS (3.0 μg/ml)-induced vasodilation (fig. 4B; n = 4; P < .05). Arteriolar diameter increased only by 5.8 ± 2.0% from baseline during suffusion of catalase (60 U/ml) and *E. coli* LPS (3.0 μg/ml).

**Role of endothelin.** PD 142893 (1 μM) and BQ-485 (1 μM) had no significant effects on *E. coli* LPS (3.0 μg/ml)-induced responses (data not shown; each group, n = 4; P > .5).

**Role of prostaglandins.** Indomethacin (10 mg/kg) had no significant effects on *E. coli* LPS (3.0 μg/ml)-induced vaso-
constriction (fig. 5; P > .5). However, it curtailed E. coli LPS (3.0 μg/ml)-induced vasodilation (fig. 5; each group, n = 4; P < .05). Arteriolar diameter decreased by 9.1 ± 1.2% from baseline at 4 min and by 0.3 ± 2.5% from baseline at 45 min during suffusion of E. coli LPS (3.0 μg/ml) in the presence of indomethacin (10 mg/kg).

Role of mast cells and chymase-like proteases. Sections of unexposed hamster spinotrapezius muscle and cheek pouch contain numerous perivascular mononuclear metachromatic cells with typical morphological appearance of mast cells (fig. 6, D and E, respectively). The distribution of cells with chymase-like activity (NASDCA-hydrolyzing activity) was similar to that of metachromatic cells (fig. 6, A and C). Detailed comparison of adjacent sections stained with NASDCA and toluidine blue, respectively, indicated that almost all cells containing chymase-like activity also stained metachromatically with toluidine blue (fig. 6, C and D). In nearby sections, 926 NASDCA-positive cells were enumerated in tissues containing 1705 metachromatic cells. The identity of NASDCA-cleaving activity as chymase was supported by virtual abolition of enzyme activity in tissue sections pre- and coincubated with chymostatin (fig. 6B). The proportion of mast cells exhibiting NASDCA-hydrolyzing activity was higher in the spinotrapezius muscle than in the cheek pouch. Specifically, 2928 NASDCA-positive cells were counted compared with 3218 metachromatic cells in identical regions of tissue sections. Thus, assuming that all NASDCA-positive cells are metachromatic, the percentage of mast cells that manifest chymase-like activity in the spinotrapezius muscle and cheek pouch is 91% and 54%, respectively. The number of NASDCA-positive cells was significantly lower in the spinotrapezius muscle of E. coli LPS-exposed (fig. 6F) than unexposed (fig. 6E) hamsters (data not shown).

Chymostatin (10 μg/ml) significantly attenuated E. coli LPS (3.0 μg/ml)-induced immediate biphasic vasomotor response (fig. 7A; each group, n = 4; P < .05). Arteriolar diameter decreased by 3.3 ± 1.2% from baseline at 4 min and increased by 5.3 ± 2.1% from baseline at 45 min during suffusion of chymostatin (10 μg/ml) and E. coli LPS (3.0 μg/ml). Soybean trypsin inhibitor (100 μg/ml) had similar effects on E. coli LPS (3.0 μg/ml)-induced responses (fig. 7B; each group, n = 4; P < .05). Arteriolar diameter decreased by 4.4 ± 1.0% from baseline at 4 min and by 2.4 ± 1.5% from baseline at 45 min during suffusion of soybean trypsin inhibitor (100 μg/ml) and E. coli LPS (3.0 μg/ml). In hamsters pretreated with compound 48/80, E. coli LPS (3.0 μg/ml)-induced vasoconstriction was abrogated, whereas vasodilation was converted to vasoconstriction (fig. 8; each group, n = 4; P < .05). Arteriolar diameter increased by 2.3 ± 1.0% from baseline at 4 min and decreased by 13.5 ± 5.7% from baseline at 45 min during suffusion of E. coli LPS (3.0 μg/ml). Intraperitoneal injection of saline for 4 days had no significant effects on E. coli LPS (3.0 μg/ml)-induced responses (fig. 8; each group, n = 4; P > .5).
Chymostatin (10 μg/ml) and soybean trypsin inhibitor (100 μg/ml) had no significant effects on AT II (0.05 μM)-induced vasoconstriction. Arteriolar diameter decreased by 22.7 ± 1.9%, 19.0 ± 2.2% and 19.6 ± 1.6% from baseline during suffusion of AT II (0.05 μM) alone (n = 9), chymostatin (10 μg/ml) and AT II (0.05 μM; n = 4) and soybean trypsin inhibitor (100 μg/ml) and AT II (0.05 μM; n = 4), respectively (P > .5).

Role of other proteases. Lisinopril (10 μM) and a mixture of leupeptin, Bestatin and DL-2-mercaptomethyl-3-guanidinoethylthiopropanoic acid (each, 10 μM) had no significant effects on E. coli LPS (3.0 μg/ml)-induced changes in arteriolar diameter (fig. 9, A and B, respectively; each group, n = 4; P > .5).

Discussion

This study presents two new findings. First, suffusion of E. coli LPS on the in situ hamster spinotrapezius muscle, at concentrations similar to circulating levels in sepsis syndrome (Shenep and Morgan, 1984; Shenep et al., 1988), for 60 min elicits an immediate biphasic vasomotor response, vasoconstriction followed by vasodilation. This response is not related to nonspecific damage to microvascular endothelium because arteriolar diameter returns to baseline once suffusion of E. coli LPS is stopped. Second, E. coli LPS-induced vasoconstriction is abrogated by SK&F 108566, a selective, nonpeptide AT, RA, chymostatin and soybean trypsin inhibitor. These compounds also attenuate E. coli LPS-induced vasodilation. By contrast, SOD, catalase and indomethacin attenuate E. coli LPS-induced vasodilation and have no significant effects on E. coli LPS-induced vasoconstriction. Endothelin receptor antagonists and the protease inhibitors lisinopril, leupeptin, Bestatin and DL-2-mercaptomethyl-3-guanidinoethylthiopropanoic acid are ineffective.

Histochemical analysis of hamster spinotrapezius muscle and cheek pouch reveals abundant perivascular mast cells with chymostatin-inhibitable chymase-like activity. Pretreatment of hamsters with compound 48/80 for 4 days to deplete mast cell of preformed mediators, including chymase-like protease(s) (Gao et al., 1993; Huntley et al., 1985; Li et al., 1993; Raud, 1989; Rubinstein et al., 1990; Shepherd and Duling, 1996; Urbaschek and Urbaschek, 1979), curtails E. coli LPS-induced vasoconstriction and converts vasodilation to vasoconstriction in the spinotrapezius muscle. On balance, these data indicate that E. coli LPS stimulates perivascular mast cells in the hamster spinotrapezius muscle to release an AT II-producing chymase-like protease(s). Angiotensin II thus produced elicits local vasoconstriction and elaborates reactive oxygen species which, in turn, generate vasodilators, prostaglandins. A proposed mechanism by which E. coli LPS modulates immediate biphasic vasomotor dysfunction in the in situ hamster spinotrapezius muscle is depicted schematically in figure 10.

The hamster is an established model to elucidate mechanisms underlying the deleterious effects of E. coli LPS and preformed mast cell mediators in the in situ peripheral microcirculation (Li et al., 1993; Shepherd and Duling, 1996; Svensjö et al., 1990; Urbaschek and Urbaschek, 1979). Urbaschek and Urbaschek (1979) showed that suffusion of E. coli LPS on the in situ cheek pouch, at concentrations similar to those used in this study, elicits transient vasoconstriction and mast cell degranulation. However, the mechanisms underlying E. coli LPS-induced vasoconstriction were not elucidated. It is well established that chymase, a preformed mast cell serine protease, is released upon mast cell degranulation and elaborates AT II in the tissue (Huntley et al., 1985; Husain, 1993; Martin et al., 1992; Okamura et al., 1990; Pearce et al., 1985; Reilly et al., 1982; Takai et al., 1996; Wintroub et al., 1984). To this end, Cornish et al. (1979) identified an ACE-independent metabolic pathway(s) in the in situ cheek pouch that produces AT II. An AT II-generating chymase-like protease was detected recently, and purified from cheek pouch homogenates, although its cellular origin(s) was not determined (Takai et al., 1996). This protease was inhibited by chymostatin and soybean trypsin inhibitor and not by ACE inhibitors (Takai et al., 1996).

The results of this study support and extend these observations by showing that relatively large numbers of perivascular mast cells containing AT II-producing chymase-like protease(s) are present in the hamster spinotrapezius muscle. This protease(s) is likely to play a role in modulating E. coli LPS-induced immediate biphasic vasomotor dysfunction.
in the muscle microcirculation because chymostatin and soy-
bean trypsin inhibitor, two relatively selective and potent
chymase inhibitors (Martin et al., 1992; Reilly et al., 1982)
and SK&F 108566, a selective nonpeptide AT1 RA, abrogate
E. coli LPS-induced responses. These effects are specific be-
cause inhibitors of other AT II-forming proteases, including
ACE, have no significant effects on E. coli LPS-induced re-
sponses, and because chymostatin and soybean trypsin in-
hibitor have no significant effects on vasoconstriction elicited
by exogenous AT II in the in situ spinotrapezius muscle. Deple-
tion of mast cells from preformed mediators with com-
pound 48/80 curtails E. coli LPS-induced vasoconstriction
and converts vasodilation to vasoconstriction.

Pretreatment of hamsters with compound 48/80 may also
release histamine and tryptase which are packed together
with chymase in mast cell granules (Martin et al., 1992; Raud, 1989; Rubinstein et al., 1990; Shepherd and Duling,
1996). Conceivably, both phlogistic mediators could modulate
E. coli LPS-induced responses in the in situ hamster spin-
trapezius muscle. However, this possibility seems unlikely
because tryptase, unlike chymase, is not inactivated by soy-
bean trypsin inhibitor (Martin et al., 1992; Rubinstein et al.
1990; Takai et al., 1996), which abrogates E. coli LPS-in-
duced responses in the spinotrapezius muscle, and because
histamine elicits immediate vasodilation in the in situ per-
ipheral microcirculation of hamsters (Raud, 1989). Taken
together, these data suggest that E. coli LPS stimulates
perivascular mast cells in the in situ hamster spinotrapezius
muscle to release an AT II-producing chymase-like pro-
tease(s). However, the cellular origin(s) of AT II produced in
the muscle was not elucidated. Additional studies are war-
ranted to address this issue.

Current concepts suggest that generation of reactive oxy-
gen species is amplified in sepsis syndrome and contributes
to vasomotor dysfunction, partly by eliciting potent vasodila-
tion in the peripheral circulation (McKenchnie et al., 1986;
Natanson, 1994). The results of this study support this no-
tion. We found that AT II produced in the in situ hamster spinotrapezius muscle during suffusion of E. coli LPS elabo-
rates superoxide and hydrogen peroxide. These mediators, in
turn, activate cyclooxygenase to generate vasodilator prostag-
landins because indomethacin, at a concentration known to
inhibit cyclooxygenase in hamsters (Gao et al., 1993, 1995;
Raud, 1989; Rubinstein et al., 1991), abrogates E. coli LPS-
induced vasodilation without affecting the initial vasocon-
striction (Feng et al., 1995; Gao et al., 1995; Warren et al.,
The magnitude of vasodilation elicited by prostaglandins in the in situ hamster spinotrapezius muscle during suffusion of E. coli LPS corresponds to ~50% reduction in peripheral vascular resistance. This figure is consistent with that observed in patients with sepsis syndrome (Hess et al., 1981; Natanson, 1994). Overall, these data suggest that E. coli LPS-induced release of chymase-like protease(s) from perivascular mast cells in the in situ skeletal muscle activates a local cascade of biologic responses leading to AT II-dependent production of reactive oxygen species, PGs, prostaglandins.

SK&F 108566, a selective, nonpeptide AT1 RA (Edwards et al., 1991), abrogates E. coli LPS-induced vasodilation in the in situ hamster spinotrapezius muscle. Because this compound also inhibits the initial E. coli LPS-induced vasoconstriction, the subsequent blockade of vasodilation could reflect the lack of stretch-induced, nitric oxide-mediated vasodilation (Natanson, 1994; Warren et al., 1991; Wurster et al., 1994). This possibility seems unlikely, however, because Gao et al. (1995) showed that N\textsuperscript{\textomega}-L-nitro arginine, a nitric oxide synthase inhibitor, has no significant effects on E. coli LPS-induced immediate biphasic vasomotor dysfunction in the in situ hamster cheek pouch.

Resident and migrant cells and phlogistic mediators other than perivascular mast cells and AT II-producing chymase-like protease(s) could play a role in modulating vasomotor dysfunction in skeletal muscle microcirculation in sepsis syndrome (Natanson, 1994; Neviere et al., 1996; Shepherd and Duling, 1996; Svensjö et al., 1990; Warren et al., 1991). The results of this study support this contention partly by showing that suffusion of E. coli LPS on the in situ spinotrapezius muscle of hamsters depleted of mast cell chymase-like pro...
hese(s) by compound 48/80 still elicits vasoconstriction. However, this response is slower to evolve than that observed during suffusion of E. coli LPS in saline-treated hamsters. The putative role of other cells and phlogistic mediators in modulating E. coli LPS-induced immediate vasoconstrictor dysfunction in situ hamster spinotrapezius muscle should be further investigated.

In summary, we found that suffusion of E. coli LPS on the in situ hamster spinotrapezius muscle for 60 min elicits an immediate, reversible biphasic vasomotor response, vasoconstriction followed by vasodilation. This response is modulated by E. coli LPS stimulation of perivascular mast cells to release an AT II-producing chymase-like protease(s). The angiotensin II thus produced elicits local vasoconstriction and elaborates reactive oxygen species which, in turn, generate vasodilator prostaglandins. We suggest that inhibitors of mast cell chymase-like protease(s) could be beneficial in the treatment of early-phase E. coli sepsis syndrome.

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References


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