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Endotoxin Releases a Substance from the Aorta that Dilates an Isolated Arteriole by Up-Regulating INOS

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Background. **Loss of vascular tone in resistance arterioles has been implicated as the cause of hypotension in septic shock. It is believed that the overproduction of nitric oxide (NO) by the inducible isoform of nitric oxide synthase (iNOS) results in the vasodilatation seen in septic shock. However, we have shown that endotoxin has no effect on vascular tone of an isolated resistance vessel unless the endotoxin flows over a segment of aorta or vena cava upstream in the superfusion line. The aim of this study was to determine if the subsequent vasodilation was due to the release of a direct vasodilator or production of NO in the arteriole and if its source was iNOS by using its selective inhibitor, aminoguanidine.**

Materials and methods. **First-order rat cremaster arterioles (n = 36) were isolated and cannulated onto micropipettes, superfused with physiological buffer at 34°C, pressurized to 70 mm Hg, and allowed to gain spontaneous tone over 90 min. A segment of abdominal aorta was then placed in series with the arteriole so that the superfusate passed over the aorta and then into the tissue bath containing the isolated arteriole. The vessels were allowed to equilibrate over 60 min. During this interval, the arteriole was exposed to L-NAME (100 μM), aminoguanidine (100 μM), or buffer. The aorta and arteriole were then superfused with endotoxin (*Salmonella enteritidis* 2.5 μg/ml). Internal diameters of cannulated arterioles were measured and recorded with videomicroscopy and videocalipers at a resolution of ±1 μm every 15 min for 1 h. Six groups were created with n = 6 for each group: Group 1, endotoxin; Group 2, control; Group 3, L-NAME and endotoxin; Group 4, L-NAME; Group 5, aminoguanidine and endotoxin; and Group 6, aminoguanidine.**

Results. **After the 60-min equilibration period, there was no significant difference in resting tone among the six groups. At t = 120, the percentage of tone in the control group was 42.7 ± 0.4% (mean ± SEM) and this was not changed by treatment with aminoguanidine (42.2 ± 0.7%). However, exposure to L-NAME alone resulted in vasoconstriction with a gain in tone to 49.5 ± 1.6% ($P > 0.05$). Endotoxin alone caused arteriolar tone to fall to 33.5 ± 1.2% ($P < 0.05$). Arterioles treated with aminoguanidine did not lose tone (42.6 ± 1.7%) when exposed to endotoxin and arterioles treated with L-NAME retained their elevated tone (46.0 ± 2.2%) after treatment with endotoxin.**

Conclusions. **This study demonstrates that the aorta exposed to endotoxin releases a substance that vasodilates resistance arterioles through the up-regulation of iNOS. Aminoguanidine prevented the fall in tone following exposure to endotoxin, while use of the nonselective NOS inhibitor, L-NAME, not only blocked the fall due to endotoxin but increased basal tone by blocking the constitutively active eNOS.**

Key Words: **endotoxin; L-NAME; aminoguanidine; sepsis; shock; vascular tone; nitric oxide; nitric oxide synthase.**

INTRODUCTION

Sepsis remains a major cause of mortality and morbidity in intensive care units worldwide. Loss of vascular tone in resistance arterioles has been implicated as the cause of hypotension in septic shock. This hypotension can result in tissue hypoperfusion leading to multiple organ failure and possibly death. A complex cascade involving proinflammatory mediators and cy-

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tokines, including endotoxin, IL-1β, and TNF-α, is believed to be responsible for these events in a septic patient [1].

One of the key compounds believed to be involved in this process is nitric oxide (NO). NO is a membrane-permeable molecule that has multiple physiological roles including neurotransmission, regulation of platelet activity, control of vascular tone, regulation of tissue perfusion, and renal volume control. NO is produced by the conversion of L-arginine to L-citrulline by nitric oxide synthase [2]. There are three isoforms of nitric oxide synthase: neuronal, endothelial, and inducible [3]. Neuronal nitric oxide synthase (nNOS) is a constitutive protein expressed in the central and peripheral nervous systems involved in neurotransmission. Endothelial NOS (eNOS) is a constitutive protein expressed in endothelial cells that regulates vasoconstriction, blood flow, and platelet aggregation. Inducible NOS (iNOS) is an inducible protein expressed in the immune system and vasculature. In septic shock, production of iNOS occurs, resulting in large quantities of NO. It is this overproduction of NO that is believed to be an important factor in the hypotension seen with septic shock [2].

Although administration of endotoxin in an animal model results in vasodilation and shock, Glembot *et al.* made the surprising discovery that endotoxin was without effect on the spontaneous tone of isolated resistance vessels, the major contributors to peripheral resistance and regulation of blood pressure [4]. However, when the isolated skeletal muscle arterioles were placed in-series with a segment of aorta and exposed to endotoxin, they demonstrated a significant loss of tone that began after a 30-min delay and progressed over the next 30 min. The vasodilatory substance released by the aorta was not blocked by indomethacin or removal of the endothelium [4] but inhibitors of NF-κB prevented the subsequent vasodilatation [5]. As NF-κB mediates the inflammatory response, this finding along with the delayed response suggests the possibility that induction of iNOS was responsible for the loss of tone.

In the present study, we tested the hypothesis that iNOS in the arteriolar wall was responsible for the loss of tone following exposure of the aorta-arteriole model to endotoxin. NOS was inhibited in the arteriole alone by two compounds, N^{ω}-nitro-L-arginine methyl ester (L-NAME) and aminoguanidine. L-NAME is an analogue L-arginine that inhibits both eNOS and iNOS in a nonselective manner [6]. Alternatively, aminoguanidine selectively inhibits iNOS with a 10- to 100-fold greater affinity for iNOS than for eNOS [7–9]. By using these two compounds, a comparison can be made between nonselective and selective inhibition of NOS with better characterization of the roles of eNOS and iNOS in our model of septic shock. The results indicate that, while eNOS is constituently active, iNOS is the isoform responsible for loss of vascular tone in our model of septic shock.

MATERIALS AND METHODS

Protocols were approved by the Animal Care and Use Committee at Eastern Virginia Medical School. All experimental procedures were in accordance with the *Guiding Principles in the Care and Use of Animals* approved by the Council of the American Physiological Society, and with federal laws and regulations.

Experiments and Methods

Male Sprague Dawley rats (n = 36) weighing 200–350 g were used in these studies. The rats were housed in a dedicated animal facility with free access to rat chow and drinking water and exposed to a 12/12-h light/dark cycle. The rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (60 mg/kg).

Preparation and isolation of the arteriole and aortic segment. To determine the influence of upstream arterial conduit vessels on skeletal muscle arterioles during exposure to endotoxin, a segment of a first-order cremasteric arteriole and a segment of abdominal aorta were prepared. An incision was made on the ventral surface of the scrotal skin to expose the cremaster muscle surrounding the testis. The cremaster was then excised at the most proximal extent of the inguinal canal and placed in a dissecting chamber filled with cold (4°C) Krebs-bicarbonate-HEPES buffer solution (119 mM NaCl, 4.7 mM KCl, 1.17 mM $MgSO_4$, 1.6 mM $CaCl_2$, 22 mM $NaHCO_3$, 1.18 mM KH_2PO_4, 8 mM HEPES, 5 mM glucose) at pH 7.4. The cremaster was pinned flat and, under a dissecting microscope, a 2-mm segment of first-order cremasteric arteriole was isolated, transferred to a tissue bath, and cannulated with micropipettes (Fig. 1). The vessel was secured to the pipettes with 10-O monofilament suture. The tissue bath was moved to a Nikon inverted microscope coupled to a charge-coupled device videocamera and a high-resolution monitor (Fig. 2). The arteriole was then pressurized to 70 mm Hg in the absence of intraluminal flow via an adjustable pressure reservoir [10]. The vessel was warmed to 34°C, the *in situ* temperature of the rat cremaster, by continual infusion of the buffer solution at rate of 4 ml/min. The buffer was bubbled with 95% N_2/5% CO_2 gas resulting in a pO_2 of 60 mm Hg at the level of the vessel. At this point, the arteriole was allowed to achieve spontaneous basal tone over 60 to 90 min. To be acceptable for the study, the arterioles had to be free of all pressure leaks and demonstrate spontaneous tone, an inherent ability of the vessel to constrict independent of neuroendocrine factors.

After achievement of spontaneous tone, a 1-cm segment of abdominal aorta was placed in a flow-through chamber connected in series to the arteriole, allowing the superfused Krebs-bicarbonate-HEPES

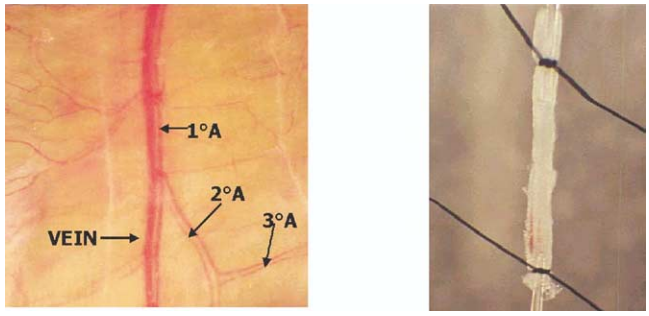

FIG. 1. The cremaster muscle is supplied by a paired artery and vein that branch. The cremaster muscle pinned flat under a dissecting microscope. A 2-mm segment of arteriole cannulated and secured with 10-O monofilament. (Color version of figure is available online.)