

**PATHOPHYSIOLOGY OF ENDOTOXIN:
MICROVASCULAR DYSFUNCTION,
AND THE ROLES OF VEGF AND NITRIC OXIDE (NO)**

A Senior Honors Thesis

by

MARK ANDREW NAFTANEL

Submitted to the Office of Honors Programs
& Academic Scholarships
Texas A&M University
In partial fulfillment of the requirements of the

UNIVERSITY UNDERGRADUATE
RESEARCH FELLOWS

April 2001

Group: Biomedical Sciences

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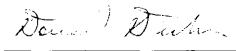
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Approved as to style and content by:



Janet Parker
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April 2001

Group: Biomedical Sciences

ABSTRACT

Pathophysiology of Endotoxin:

Microvascular Dysfunction,

And the Roles of VEGF and Nitric Oxide (NO). (April 2001)

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Vascular endothelial growth factor (VEGF) elicits nitric oxide (NO)-dependent vasodilation and plays major roles in angiogenesis and wound healing. Although bacterial endotoxin (LPS) has been shown to alter endothelial NO synthase (eNOS)/NO regulation, interactive effects of LPS and NO-dependent responses to VEGF have not been examined. We compared microvascular endothelium-dependent relaxation (EDR) to VEGF¹⁶⁵ (10^{-14} - 3×10^{-11} M) in mesenteric microvessels ($\sim 150 \mu\text{m}$) isolated from control (sterile saline-treated) and endotoxemic (*E. coli* LPS; 4 mg/kg i.p., 16 hrs) guinea pigs. Specialized microvessel myographs and isometric techniques were used to study EDR to VEGF following pre-contraction of vessels with endothelin-1 ($\sim \text{EC}_{50}$). Microvessels isolated from control animals, but not LPS-treated animals, exhibited significant concentration-dependent EDR to VEGF; vasorelaxation (% endothelin precontraction) at 3×10^{-11} M VEGF averaged $63 \pm 17\%$ and $8.1 \pm 9.9\%$, respectively. Furthermore, exposure to the NO synthase inhibitor L-NMMA ($100 \mu\text{M}$) significantly blocked vasorelaxation to VEGF ($< 3 \times 10^{-11}$ M) of control microvessels, but produced no effect on microvessels from

LPS-treated animals; relaxation at 10^{-11} M VEGF averaged $4.4 \pm 18\%$ and $-11 \pm 11\%$, respectively. Thus, these data indicate that *in vivo* endotoxemia impairs NO-dependent *in vitro* microvascular relaxation to VEGF. Since NO plays a major role in VEGF-stimulated vasodilation, permeability, proliferation, and angiogenesis, these data may implicate LPS-induced impairment of VEGF-stimulated NO production in the endothelial pathophysiology of shock/sepsis.

DEDICATION

I would like to dedicate this project to my parents, John and Sue Naftanel. I cannot imagine two more loving and supportive people, and I thank God for blessing me with such a wonderful family. They have been able to achieve the rare balance of providing unconditional support, while also providing me the freedom to develop as an individual. I took this for granted earlier in my life, but now I realize how fortunate I am. Whether it was helping me with homework or traveling across the nation to various diving meets, my parents were always there for me. Any successes that I have had in the past or may have in the future are a direct result of Mom and Dad's support.

ACKNOWLEDGMENTS

I feel extremely blessed to have worked with many wonderful people throughout this past year. Without their assistance and support, this project would not have been possible. First of all, I would like to express my sincere appreciation to everyone in Dr. Janet L. Parker's lab in the Department of Medical Physiology at the Texas A&M College of Medicine. I began my association with this lab in the spring of 1999 as a student worker, and I later asked Dr. Parker if she would serve as my mentor for the Undergraduate Research Fellows program. She eagerly said yes, and I am very fortunate that she did. Dr. Parker has served as a tremendous advisor on this project, providing me with all of the information and material resources imaginable. I would also like to acknowledge the American Heart Association, who has funded Dr. Parker's shock research and made my project possible. In addition to helping with this specific project, Dr. Parker has also given me a lot of insight and advice on my future career in medicine. I will always be grateful for all the help she has given me.

The other members of the Parker lab have also been instrumental during the course of this project. Their years of experience and expertise, combined with their willingness to help, allowed me to conduct research in a very supportive environment. Millie Mattox, Associate Research Specialist, provided invaluable information and technical assistance in every phase of this project. She performed the required surgeries, prepared the vessels, demonstrated the myograph technique, assisted in data analysis, and helped out in many other areas whenever I needed help. Without her, I would not have known where to begin, and I truly appreciate all the time she spent with me that took away from her regular lab

duties. Beth Becker, a Research Assistant in Dr. Parker's lab, has also been a tremendous resource during this past year. She is a wonderful woman who always took extra time to thoroughly explain new concepts to me in the lab. She has become an expert on the nitric oxide analyzer, and I enjoyed working with her as she set up the new equipment. Paige Charlebois began as a student worker in Dr. Parker's lab and recently started full-time in January following graduation. We have had a lot of laughs and good times in the lab, and I thank her for making my time here so much fun. Paige also served as a "guinea pig" for my presentations, patiently sitting through slide show after slide show and giving me very helpful feedback. I wish Paige and her soon-to-be husband Chris the very best in the future. Finally, Jennifer Fogarty is a doctoral student in Dr. Parker's lab, and she often clarified things for me and gave me advice throughout the various stages of this project. Jen, despite her questionable opinion of AggieLand, was great to work with and will undoubtedly succeed in whatever career she decides to pursue.

In addition to the members of the Parker lab, I would also like to thank the Office of Honors Programs & Academic Scholarships. In particular, Dr. Donald Dickson was very helpful during the application and proposal process. Since I spent the year prior to my Fellows year in Austin, it was a challenge to coordinate the logistics of submitting a proposal. Dr. Dickson made sure I had all the necessary information and assisted me throughout the process. Also, my friend Meghan Zack served as my courier in College Station by running all over campus getting the required signatures and submitting the final product. Thank you for all your help.

TABLE OF CONTENTS

	Page
ABSTRACT	iii
DEDICATION	v
ACKNOWLEDGMENTS	vi
TABLE OF CONTENTS	viii
NOMENCLATURE	ix
LIST OF FIGURES	x
INTRODUCTION	1
METHODS AND MATERIALS	8
Subjects	8
Endotoxemia Model	8
Vessel Collection and Preparation	9
Solution and Drugs	11
Concentration-Response Curves	11
Data Analysis and Statistics	12
RESULTS	14
Acetylcholine	14
VEGF	16
CONCLUSION	18
REFERENCES	21
VITA	23

NOMENCLATURE

ACh \Rightarrow Acetylcholine

ANOVA \Rightarrow Analysis of Variance

EDR \Rightarrow Endothelium-derived relaxation

L-NMMA \Rightarrow N^G monomethyl-L-arginine (L-NMMA)

NO \Rightarrow Nitric Oxide

ecNOS \Rightarrow constitutive nitric oxide synthase

iNOS \Rightarrow inducible nitric oxide synthase

VEGF \Rightarrow Vascular Endothelial Growth Factor (also known as vascular permeability factor)

LIST OF FIGURES

Figure		Page
1	Endothelium-mediated vascular relaxation to agonists: inhibition by L-NMMA	4
2	Endothelium-mediated vascular relaxation to agonists	6
3	VEGF signaling in endothelium	6
4	Dissection and mounting of mesenteric arteries	10
5	View of myograph	10
6	ACh dose-response curve, no pre-treatment	13
7	ACh dose-response curve, 100 μ L L-NMMA	14
8	VEGF dose-response curve, no pre-treatment	15
9	VEGF dose-response curve, 100 μ L L-NMMA	16

INTRODUCTION

Septic Shock

Septic shock (also known as endotoxic shock) is a major cause of illness and death among hospitalized patients. Despite extensive research, septic shock still results in over 100,000 deaths annually, making it the leading cause of death in surgical intensive care units (1). Research in the last few decades has increased the understanding of septic shock's underlying pathophysiological mechanisms and improved the treatment of septic patients, but the mortality rate due to septic shock remains alarmingly high (2). Therefore, it continues to be an area of intense research with profound clinical implications.

In the early stages of sepsis and shock, patients show general signs of bacterial infection (e.g. fever; increased circulation). As the infection progresses, plasma is lost into infected tissues through "leaky" capillary walls, reducing overall blood pressure. At a certain point, the deterioration of circulation becomes progressive like all other types of shock, in which the cardiovascular system itself – heart, blood vessels, vasomotor system – begins to break down, so that the shock state becomes progressively worse (3). Shock ultimately results in hypotension (low blood pressure) associated with indications of poor tissue perfusion, such as decreased urine production, reduced skin perfusion, and alteration in mental alertness (4). Low blood pressure and the concomitant insufficient blood flow cause various organ dysfunctions, which include reduced alertness, hypoxemia, increased airway resistance, reduced protein synthesis, increased bilirubin levels, and impaired glucose metabolism (5).

This thesis follows the style and format of *Shock*

Vascular dysfunction during septic shock is characterized by a heterogeneous mixture of peripheral vasodilation and vasoconstriction in different organs, resulting in decreased systemic vascular resistance, regional blood flow insufficiency, and tissue ischemia (6,7). In addition to its effects on vascular resistance, it is now recognized that septic shock can also lead to myocardial depression. This is more difficult to diagnose because cardiac output (product of heart rate and stroke volume) is usually high or normal in septic patients, particularly in the early stages. The observed increased cardiac output is a result of the neurohumoral compensatory mechanisms (i.e. tachycardia) seen in sepsis; these mechanisms tend to mask the decreased contractile function caused by the depression of cardiac muscle (4).

Lipopolysaccharide (LPS)

A major mediator of septic shock is bacterial lipopolysaccharide (LPS), also known as endotoxin. *Escherichia coli* (*E. coli*) is the most common causative agent in septic shock, and the *E. coli* cell wall component LPS is believed to mediate many pathophysiologic effects as well as elicit the release of numerous secondary mediators. Endotoxins include a variety of macromolecules with an approximate molecular weight of 200,000 that are integral membrane proteins of Gram-negative bacteria cell walls. Protein and LPS molecules are released during cell growth and cell lysis, and these materials provide the components required to synthesize endotoxin (8).

Bacterial endotoxins and their downstream mediators have profound physiological effects (9). In our current research, we focus on endotoxin's role in septic shock as it relates to its effects on the vasodilators VEGF and nitric oxide.

Nitric Oxide (NO)

Before proceeding to a discussion of nitric oxide, it is important to describe the endothelium and how it relates to vascular relaxation. All blood vessels, including arteries, arterioles, capillaries, venules, and veins, are lined with a single layer of endothelial cells. These cells form a barrier between the blood and interstitial fluid, allowing the exchange of nutrients and waste products between the blood and tissues (10). In addition, it is now clear that endothelial cells play an important role in the regulation of blood flow. Endothelial cells are especially important in the current research because many vasorelaxation effects are mediated by the endothelium.

Nitric oxide is a naturally occurring vasodilator, and because of its vascular relaxation effects, it is also known as endothelial-derived relaxing factor (EDRF). Small amounts of NO are critical for maintaining appropriate vascular tone, and it has been reported to protect against pathological vasoconstriction and organ damage during circulatory disorders (11). Under normal physiological conditions, NO is generated from L-arginine by the calcium/calmodulin-dependent enzyme, constitutive NO synthase (ecNOS), in endothelial cells. The NO diffuses into adjacent vascular smooth muscle cells and activates guanylate cyclase, producing cGMP. This in turn elicits vascular relaxation by activating a cGMP-dependent protein kinase within smooth muscle (12).

Nitric oxide production can be increased by numerous normal physiological factors, one of which is shear stress. The rapid flow of blood through arterioles increases shear stress on the endothelial cells that line the vessels, inducing an increased release of nitric oxide. The NO then diffuses into the vascular smooth muscle, producing vasodilation (13).

During sepsis, various mediators such as endotoxin and cytokines stimulate the induction of another isoform of the enzyme, inducible NO synthase (iNOS). This calcium-independent isoenzyme produces abnormally large amounts of NO, resulting in excessive vasodilation that contributes to the hypotension found in septic shock. At the same time, various vasoconstricting substances including thromboxane and endothelins are also released. As a result, there is interactive and complex competition between the antagonistic effects of vasodilation and vasoconstriction elicited by sepsis and underlying mediators.

N^G monomethyl-L-arginine (L-NMMA) is a structural analogue of L-arginine. L-NMMA thus serves as a competitive inhibitor of both NO synthase isoforms, blocking NO production (14). Therefore, we will use this NO inhibitor to evaluate NO-mediated effects of VEGF. Figure 1 below is an illustration showing how L-NMMA acts to inhibit NO synthase, resulting in a decrease in NO production and decreased vascular relaxation responses to receptor agonists

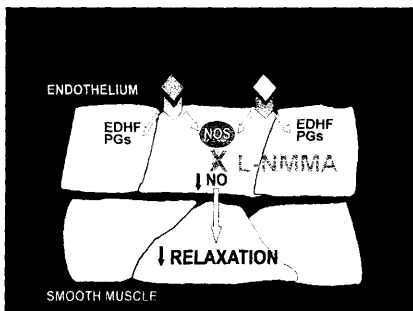


Figure 1. Endothelium-mediated vascular relaxation to agonists: inhibition by L-NMMA.

Acetylcholine (ACh)

Acetylcholine is a common neurotransmitter that activates nicotinic and muscarinic cholinergic receptors throughout the body. In the microvascular circulation, ACh acts on muscarinic receptors of endothelial cells to increase production of NO (by increasing the activity of NOS), thus causing vascular smooth muscle relaxation (15). Due to its well-documented vasodilation effect, ACh often serves as the gold standard for comparison with other endothelium-mediated vascular relaxation agonists. Figure 2 depicts the binding of ACh (or other receptor agonist) to endothelial cell receptors, which increases the levels of NO and results in vasodilation.

VEGF (Vascular endothelial growth factor)

Vascular endothelial growth factor (VEGF) is an endothelium-specific glycoprotein that enhances endothelial cell growth, increases vascular permeability, and stimulates vasodilation (16). Recent research has shown that VEGF exerts its effects through receptor-dependent pathways involving NO synthase (17, 18). With increased NOS activity, the levels of NO rise and the usual endothelium-mediated relaxation of vascular smooth muscle is seen. Figure 3 elucidates several of the VEGF pathways; we will be investigating the pathway that goes from VEGF receptor binding through eNOS to vascular smooth muscle relaxation. VEGF has also been shown to play a role in the pathogenesis of septic shock, as LPS augments VEGF expression and secretion in rat coronary vessels (19).

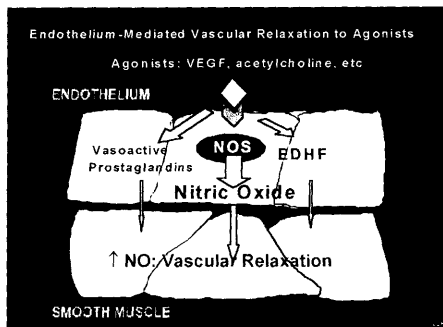


Figure 2. **Endothelium-mediated vascular relaxation to agonists.** (from J.L. Parker, modified from H. Granger and colleagues)

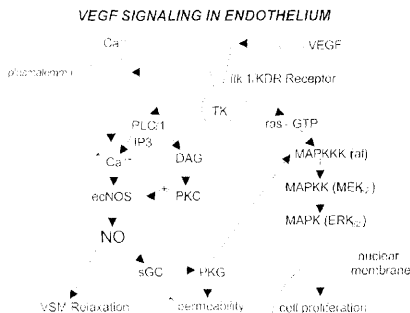


Figure 3. **VEGF signaling in endothelium.** (from J.L. Parker, modified from H. Granger and colleagues)

Current Status of Research

The last decade has witnessed an explosion of research and new knowledge in the area of septic shock. The roles of VEGF and NO are becoming more clear, but much remains to be learned. The aim of this research is to elucidate the role of VEGF in septic shock, specifically as it relates to microvascular function. The large vessels have been thoroughly studied, but it is unclear whether the microvessels operate in a similar manner. Since the microcirculation is the dominant determinant of overall blood pressure, it is important to investigate the effects that septic shock have on VEGF and NO and their interactions at this level.

MATERIALS AND METHODS

For this research project, I employed methods used by Dr. Parker's lab. These methods include the techniques for inducing *in vivo* endotoxemia in guinea pigs and *in vitro* techniques for measuring intrinsic contraction-relaxation characteristics of isolated blood vessels (20). These procedures are summarized below.

Subjects

Hartley-strain male albino guinea pigs, weighing between 250 and 400 g, were used as subjects in this study. The animals were kept at room temperature (between 22° and 24° C); a 12-hour light-dark cycle was maintained by artificial light; and experiments were timed so that blood vessel isolation began between 12:00 and 2:00PM. All animal procedures were reviewed and approved by the University Laboratory Animal Care Committee (ULACC) of Texas A&M University, College Station, TX.

Endotoxemia Model

Gram-negative endotoxemia was produced by intraperitoneal injection of 4 mg/kg purified *E. coli* endotoxin. This procedure has been shown to consistently elicit a state of circulatory shock characterized by central nervous system depression, hypothermia, \approx 20% mortality, early compensatory hyperpnea, minimal changes in arterial blood gases, and significant decreases in systolic and diastolic blood pressures by 16-18 hours after injection of endotoxin (21, 22). In the current study, blood vessels were isolated 16-18 hours after the injection of either endotoxin or an equal volume of sterile isotonic saline

solution (control vessels). Four guinea pigs were used during each trial (two control vessels, two endotoxin-exposed vessels)

Vessel Collection and Preparation

Guinea pigs were anesthetized with sodium pentobarbital (65 mg/kg) prior to decapitation, and the mesenteric arteries found in the abdominal cavity were quickly removed and placed in ice-cold bicarbonate-buffered solution. Vessels were cleaned of adipose and connective tissue, and then cut into rings with axial lengths of approximately 1.52 mm. Average vessel dimensions used in this study are presented in Table 1 below.

Table 1: Mesenteric arteries vessel dimensions.

Group	n	Outer Diameter	Inner Diameter	Wall Thickness	Axial Length
Control	14	248±10.1	162±9.2	39±1.4	1.52±.01
LPS	14	263±6.3	174±5.5	36±1.2	1.52±.01

Values are means ± SE in μm ; n, total number of vessels studied in each group

The mesenteric rings were mounted on two 20- μm tungsten wires passed through the vessel lumen (Figure 4) (23). One wire was attached to a force transducer, and the other was attached to a micrometer mounted on the transducer. This setup enabled the vascular rings to be stretched by known increments while isometric force was measured. The mounted vessels were then lowered into individual 20-mL tissue baths containing Krebs' bicarbonate solution. Figure 5 pictures the overall myograph setup (23).

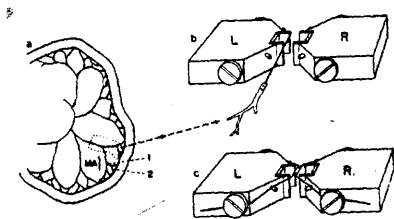


Figure 4. **Dissection and mounting of mesenteric arteries.**
(from Mulvany and Halpern, 1977)

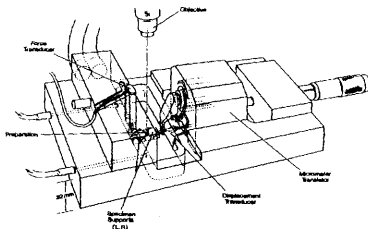


Figure 5. **View of microvessel myograph.**
(from Mulvany and Halpern, 1977)

Solutions and Drugs

The Krebs' bicarbonate buffer contained (in mM) 131.5 NaCl, 5.0 KCl, 1.2 NaH_2PO_4 , 1.2 MgCl_2 , 2.5 CaCl_2 , 25.0 NaHCO_3 , and 11.2 glucose. This solution was aerated with 95% O_2 -5% CO_2 (pH 7.35) and was maintained at 37°C. In designated solutions, the KCl concentration was increased in a substituted manner. Acetylcholine, L-NMMA, and endothelin were purchased from Sigma Chemical Company, while VEGF was obtained from R&D Systems (Minneapolis, MN). Endotoxin was purchased as lipopolysaccharide W, 0127/B8 and was prepared in sterile isotonic saline solution.

Concentration-Response Curves

After in vitro stabilization and a rapid transient stretching of each vessel three times, each mesenteric vessel was systematically stretched to the optimum of its length-active tension relation (L_{max}). We used a modified length-tension curve, with L_{max} equal to 95% of L_{100} (described below). This protocol was used because previous studies by Parker and colleagues have shown that guinea pig microvessels are optimally stretched at 95% of the determined L_{100} value. In order to determine each vessel's L_{100} , the vessels were started in their relaxed state (absence of stretch). The internal circumference (L), measured in μm , was calculated using the formula

$$L = 2f + 102.83$$

where f is the distance between wires as measured by the micrometer. Then a known amount of passive stretch was applied to each vessel, and the corresponding increase in passive wall tension (mNewtons/mm) was determined from the myograph recorder printout. The amount of stretch was increased until the plot of passive wall tension

intersected the calculated standardized plot of wall tension at 100 mm HG (L_{100}). The intersection of these two lines was taken as L_{100} , and this value was multiplied by 0.95 to generate L_{max} . All subsequent concentration response studies for vasodilators were performed with the vessels at L_{max} . In two of the four vessels in each trial, 100 μ L L-NMMA was added to the circulating baths to investigate the effects of inhibiting the nitric oxide synthase.

Prior to initiating the dose response curves, 80 mM K⁺ (substituted) was added to provide a maximum contraction for each vessel. We compared this value with the level of contraction elicited by endothelin, which was about 50% of the maximum K⁺-induced contraction. After restabilizing the vessels, endothelin (4 nM – 7nM) was added to pre-constrict the vessels. The contractile response was allowed to plateau, at which time the VEGF concentration-response curve was performed in a cumulative manner. The concentrations of VEGF used were 10^{-14} M, 10^{-13} M, 10^{-12} M, 10^{-11} M, 3×10^{-11} M, doses were administered approximately eight minutes apart. Following the maximal VEGF response, all baths were rinsed several times with Krebs' bicarbonate solution. Endothelin was added to re-constrict the vessels, after which the acetylcholine concentration-response was administered. The concentrations of ACh used were 10^{-8} M, 10^{-7} M, 10^{-6} M, 10^{-5} M, and 10^{-1} M, with each dose given after the relaxation response had plateaued (within five minutes). Relaxation responses are presented as a percentage (% relaxation) of the endothelin precontraction.

Data Analysis and Statistics

All of the traces were hand-scored to generate numerical data. The SAS statistical software was utilized to analyze and statistically compare our data. We performed a split-plot ANOVA to determine whether there was a significant difference between the shock and control vessels. Fisher's LSD mean separation test was used to compare differences between mean responses to individual doses. Data in graphs is presented as mean +/- standard error.

For acetylcholine responses (absence of L-NMMA), my data was combined with data previously collected in Dr. Parker's lab and results analyzed using pooled data from both groups. The lowest ACh dose used in the previous work was 10^{-7} M, whereas my starting dose was 10^{-8} M. As a result, the n value for the 10^{-8} M dose is four and the n value for all other doses is eight. All of the data involving L-NMMA was collected during the present study.

RESULTS

Acetylcholine

Acetylcholine produced significant relaxation responses of mesenteric microvessels isolated from both control and LPS-treated (shock) guinea pigs. However, acetylcholine relaxation was significantly impaired in mesenteric microvessels from LPS-treated animals. Figure 6 shows the relaxation responses to acetylcholine in the absence of L-NMMA treatment, presented as a percentage relaxation of the endothelin precontraction. The graph shows that there was a significant difference ($p < .01$) between the shock and control vessels' response to the three lowest doses of ACh (10^{-8} M, 10^{-7} M, and 10^{-6} M).

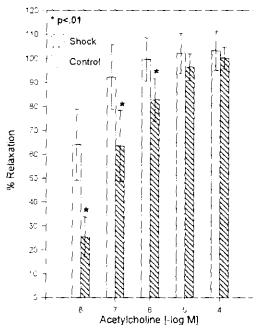


Figure 6. ACh dose-response curve, no pre-treatment.
Guinea pig mesenteric arteries, 16 hrs LPS.

Inhibition of NO synthase using L-NMMA decreased relaxation responses to acetylcholine, particularly at lower concentrations (10^{-8} M). These results are summarized in Figure 7.

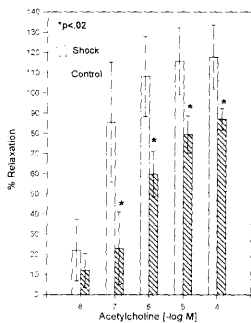


Figure 7. ACh dose-response curve, 100 μ L L-NMMA. Guinea pig mesenteric arteries, 16 hrs I.P.S.

VEGF

These results were the main focus of this research project. We ran seven separate trials, so the n value is seven for all groups except the no pre-treatment control group (which has an n value of six). As seen in Figure 8, VEGF-mediated relaxation was significantly inhibited in mesenteric microvessels isolated from LPS-treated (shock) animals as compared to microvessels from control animals. The difference between the overall concentration-response curves was significant ($p < .01$), and all but the lowest dose of VEGF were significantly different between the shock and control vessels. The relaxation response (mean \pm standard error) at the maximum VEGF administration (3×10^{-11} M) was $8.1 \pm 9.9\%$ and $63.0 \pm 17.1\%$ for the shock and control vessels, respectively.

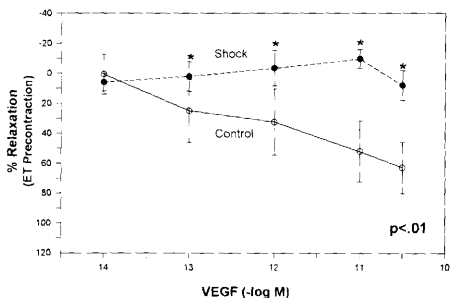


Figure 8. VEGF dose-response curve, no pre-treatment. Guinea pig mesenteric arteries, 16 hrs LPS.

Upon administration of L-NMMA, the relaxation response to VEGF was significantly reduced in the control vessels (Figure 9). However, since VEGF relaxation was already minimal in the shock group, L-NMMA produced no further inhibition of the VEGF response. Figure 9 shows that the only significant difference between relaxation responses in shock and control vessels was at the highest dose of VEGF (3×10^{-11} M).

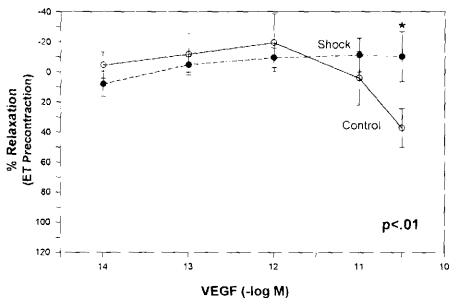


Figure 9. VEGF dose-response curve, 100 μ L L-NMMA. Guinea pig mesenteric arteries. 16 hrs LPS

CONCLUSION

Acetylcholine

Mesenteric microvessels isolated from LPS-treated (shock) guinea pigs showed significantly less relaxation to the three lowest doses of ACh as compared to control vessels. However, maximal relaxation response was not significantly different between the two groups at the highest concentrations of ACh. Thus, although microvessels from LPS-treated animals exhibited decreased sensitivity to acetylcholine, maximal relaxation was unaffected. Decreased relaxation to acetylcholine at lower concentrations (decreased sensitivity) suggests that LPS treatment may impair endothelial function and NO production in response to acetylcholine. This conclusion supports previous reports by Parker and colleagues of impaired endothelial function and decreased NO production in aorta isolated from LPS-treated guinea pigs (20, 24). One possible explanation for the unaltered maximal response is that the higher concentrations of ACh managed to increase the levels of nitric oxide to such an extent that it overcame the shock-induced impairment of vasorelaxation.

The NO synthase blocker L-NMMA inhibited acetylcholine responses of both shock and control groups and reversed the shock-induced impairment at the lower acetylcholine concentrations. However, the four highest doses of ACh elicited significantly different relaxation responses in the shock and control vessels. These results emphasize the point that an ACh-mediated increase in NO production is not the only mechanism resulting in vasodilation.

VEGF

Our results indicate that *in vivo* endotoxemia impairs NO-dependent *in vitro* relaxation to VEGF. Indeed, mesenteric microvessels isolated from control animals exhibited significant (>60%) relaxation of endothelin precontractions, whereas microvessels isolated from LPS-treated animals exhibited minimal relaxation to VEGF. These results support other lines of evidence that show agonist-stimulated nitric oxide production is impaired in septic shock (20,24). To our knowledge, no other study has evaluated effects of LPS upon microvascular responses to VEGF. Our results are particularly significant because the microvasculature is primarily responsible for vascular tone and thus is the major determinant of systemic blood pressure. Also, since NO plays a major role in VEGF-stimulated dilation, permeability, proliferation, and angiogenesis, these data may implicate impaired VEGF-stimulated NO production in the pathophysiology of shock/sepsis.

Our data using the inhibitor of NO synthase provided functional data suggesting that the mechanism underlying LPS-induced impaired VEGF responsiveness involves decreased NO synthesis/production. The next logical step in this line of study is to investigate the direct production of nitric oxide upon administration of VEGF. Dr. Parker's lab has recently acquired a sophisticated nitric oxide analyzer that will permit the direct on-line measurement of nitric oxide via chemiluminescence techniques. However, the operation of this equipment is quite complex, and it has required some trial and error to perfect the procedure. After these initial problems are worked through, we will be able to take cultured endothelial cells or isolated microvessels and directly measure the amount of nitric oxide given off by these cells. Our current study indicates that VEGF exerts its

vasodilation effects via a nitric oxide pathway and that exposure to LPS inhibits this pathway. The added line of evidence with direct measures of NO and cultured cells would serve to support this finding.

Throughout the project, several minor problems arose which had to be dealt with. First of all, the very nature of VEGF and its complex intracellular pathways result in a slow-acting vascular smooth muscle relaxation response. VEGF utilizes a tyrosine kinase second messenger system, so vasodilation effects are not seen immediately. On the other hand, acetylcholine acts much more quickly on the vascular smooth muscle, so its effects are more readily seen. Another complication in this study was the possibility that the endothelin continued to vasoconstrict the vessels after the dose-response curve had been started. I paid special attention to wait for the vessels to reach a steady level of constriction, but some of the vessels never completely leveled off. Therefore, it is possible that some of the vasodilation responses to VEGF and/or ACh may have been masked by the continued endothelin-mediated vasoconstriction. This, along with the slow-acting nature of VEGF, would have resulted in an under-estimation of the actual relaxation caused by VEGF and/or ACh. Finally, we had one very odd response to VEGF administration in one of our trials. On the 12/5/00 trial, the administration of VEGF was followed by a distinct contraction response, and even the highest doses of VEGF did not elicit any relaxation. These results did not match our other data, but we could not think of a good reason for why this occurred. As a result, we included this data in the statistical analyses, and we still had significant differences between the shock and control groups.

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VITA

Mark Andrew Naftanel

Academics

Texas A&M University, College Station, TX (1995-2001)

*B.S. in Biomedical Science and Psychology

*Current 3.93 / 4.0 GPA

*Graduation in May 2001 with University and Foundation Honors

*President's Endowed Scholarship recipient

Stephen F. Austin High School, Austin, TX (1991-1995)

*Class of 1995 valedictorian

*National Merit Scholar

Activities

Texas A&M University Undergraduate Research Fellows Program

*First Place, Oral Undergraduate Biological Sciences II, 2001 TAMU Student Research Week

Publications

*Abstract "Pathophysiology of endotoxin: microvascular dysfunction, and the roles of VEGF and nitric oxide (NO) (in press) - to be presented at 2001 Shock Society Conference.

Awards and Honors

*Who's Who Among Students in American Universities and Colleges for 1998-1999

*1999 Buck Weirus Spirit Award (honoring 40 A&M students who have been active leaders)

*Dean's List (1996, 1997, 1998, 1999, 2001)

*Phi Eta Sigma National Honor Society (1996 - 1999)

*Phi Kappa Phi National Honor Society (1997 - 1999)

*1998 Texas A&M Male Scholar-Athlete of the Year

*1997, 1998, 1999 NCAA Academic All-American

Texas A&M Swimming and Diving Team

*Seven-time NCAA All-American

*Seven-time Big XII Conference Diving Champion

*1997, 1998, 1999 Big XII Conference Diver of the Year

*A&M school record-holder in every diving event

*Member of the 1998, 1999, and 2000 U.S. Diving National Teams

*2000 U.S. Olympic Trials semi-finalist

Aggie Athletes Involved

*Active member of this community service organization that volunteers throughout the community.

Student Athlete Advisory Committee

*Chosen to serve on this committee which acts as a liaison between athletes and the Athletic Dept.

*Served as the Committee Chair in 1998-1999

Career & Educational Goals

After graduation from Texas A&M University, I will matriculate at Baylor College of Medicine to pursue my M.D. degree.

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