

SPLANCHNIC VASCULAR CONTROL DURING SEPSIS AND ENDOTOXEMIA

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1. ABSTRACT

Endotoxemia and sepsis often result in circulatory derangements which manifest as perfusion maldistributions. It has been widely accepted that the splanchnic circulation decreases in perfusion during advanced septic or endotoxemic states. Impaired perfusion of splanchnic organs may result not only in organ dysfunction but also exacerbations of polymicrobial bacteremia due to intestinal mucosal leakage. Consequently, evaluation of the splanchnic mechanisms of vasoregulation and how perfusion is maintained is vital to any topic concerning the management of the septic patient.

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2. INTRODUCTION

2.1 Endotoxemia, sepsis and septic shock

Despite numerous studies and clinical interventions, sepsis remains a leading cause of morbidity and mortality in intensive care units (1,2) and is the leading cause of death in non-cardiac surgical services (3). From the description of a "passively transferable lethal factor" in the blood of rats during hemorrhagic shock (4) to the discovery of nitric oxide as a potent effector of vasodilation and vascular unresponsiveness (5), many ideas have evolved over the years to explain the changes in vascular tone during shock and sepsis.

Inherently associated with the lethality of sepsis is the development of the "Systemic Inflammatory Response Syndrome" resulting in progression to multi-system organ failure. Homeostatically and also initially during the progression of sepsis, the intestinal mucosa functions as a major local defense barrier that helps prevent dissemination of bacteria and endotoxin normally

contained within the lumen. Since the gastrointestinal (GI) tract represents the major endogenous source of endotoxin, much research has been focused on the role of the splanchnic organs and its circulation and/or function during sepsis.

This review will describe: 1) various experimental models of producing endotoxemia and/or sepsis; 2) elucidate issues regarding vascular control in septic states with particular emphasis on the splanchnic vasculature; and 3) describe interventions clinically and experimentally which may result in improved vascular perfusion to the splanchnic organs.

3. ANIMAL MODELS: HEADING TOWARDS A CLINICALLY RELEVANT MODEL?

In studies assessing physiologic changes related to septicemia, the decision regarding the model of inducing the septic state must be addressed. Although often used synonymously, differences exist between the induction of endotoxemia and the induction of sepsis. In general, for a particular animal model, endotoxemia induction results in a more direct, reproducible alteration in host responses to the immune challenge. Sepsis induction, although less predictable and less reproducible, results in a situation more akin to that seen clinically.

3.1 Endotoxemia

Endotoxin is located in the cell wall of gram negative bacteria and is responsible for the systemic alterations seen in the shock state. Referred to as the O-somatic antigen, endotoxin may successfully be removed from the cell wall with trichloroacetic acid. Protective effects of monoclonal antibodies against the lipid-A component were demonstrated *in vivo* (6), yet clinical trials have been viewed with disappointment (7).

Endotoxemic animals exhibit changes that are species-specific. In pigs and young equines pulmonary hypertension with associated lung injury are commonly observed (8-12). In dogs and rodents, the GI tract is the principal affected organ without the development of significant pulmonary hypertension (13,14). Sensitivities to endotoxin challenges also vary among species. Pigs possess a significant sensitivity to endotoxin, as low doses ($<5\mu\text{g/kg}$) induce marked cardiopulmonary effects (15). Dogs and rats (13,14) may tolerate extremely high doses (1 mg/kg) whereas extreme sensitivity is seen with guinea pigs as marked effects occur with the administration of less than 1 $\mu\text{g/kg}$ (16). For comparison, endotoxin doses of 4 ng/kg induces a pyretic response and shock-like hemodynamic changes in healthy human volunteers (17).

Animals injected with endotoxin experience hypotension and yet an increased peripheral resistance (18). Both cardiac output and mean pressure fall and an adrenergic discharge results, which raises peripheral resistance (19). Transient hyperglycemia due to glycogenolysis followed by a severe hypoglycemia ensues as glycogen stores become exhausted and hepatic gluconeogenesis is inhibited (20-24).

3.2 Sepsis

Induction of the septic state inherently is more challenging than inducing endotoxemia since the immune challenge is episodic in nature. Wichterman *et al* in their review of septic modeling outlined the following guidelines (partially presented here) for progressive and lethal septic models which continues to hold true: (1) The animals should show clinical signs of sepsis (malaise, fever, chills, generalized weakness); (2) The septic insult should occur over a period of time to allow the animal time to respond to the insult and attempt to overcome it; (3) The model should be reproducible enough so that at least the majority of the prepared animals are available for study (25).

The major methods utilized lately to achieve this experimentally have been: (1) intravenous infusion of live bacteria; (2) surgical disruption of cecal mucosal integrity; and (3) administration of live organisms into the peritoneal cavity via a cecal slurry. The bolus intravenous infusion of live *Escherichia coli* microorganisms (26) does not accurately simulate the human situation due to the abruptness of the immune challenge, and since clinically, patients experience intermittent release of toxin into the blood stream from a septic focus. In an effort to achieve a model similar to the situations faced by clinicians, a method which results in an episodic release of organisms into the bloodstream would be desirable. Since individuals with identical septic insults may respond in totally different fashions, this physiologic variability would also be expected to be observed in those models deemed "most clinically relevant".

Two methods, popularly utilized to induce sepsis in experimental animals, are cecal ligation and puncture (25) and fecal pellet implantation (27). Cecal ligation and puncture (CLP) has been utilized with acceptable results since its description by Wichterman *et al* in 1980 (25). As a model of sepsis, it satisfies the requirement of episodic release of microorganisms into the bloodstream. However, this model produces a rapidly lethal septic state with mortality varying from near 100% at 16-24 hrs to 77% at five days depending on the surgical technique,

Table 1. Changes in physiologic parameters associated with abdominal cecal slurry model of sepsis

	MAP	PP	HR	WBC	LACTATE	% Wt.
Pre-Sepsis	116 ± 4.9	31.5 ± 1.4	355 ± 13	8.1 ± 0.6	0.6 ± 0.05	N/A
2 Hours	99 ± 5.4*	39.0 ± 2.6*	359 ± 10	ND	ND	N/A
4 Hours	103 ± 6.1	40.0 ± 2.9*	390 ± 10	ND	1.56 ± 0.1*	N/A
24 Hours	104 ± 5.8	38.6 ± 5.9	457 ± 21*	2.5 ± 0.2*	1.2 ± 0.8*	-5.46
48 Hours	110 ± 4.4	47.5 ± 3.6*	416 ± 24	8.4 ± 0.5	ND	-12.6
72 Hours	101 ± 4.4	45.0 ± 8.4*	409 ± 13	12.7 ± 1.2*	2.5 ± 0.9*	-10.13
7 Days	91 ± 2.7*	23.8 ± 5.4*†	449 ± 7*	16.0 ± 1.2*†	5.6 ± 1.5*†	-3.58

Parameters include mean arterial blood pressure (MAP; mmHg), pulse pressure (PP; mmHg), heart rate (HR; beats/min), white blood cell count (WBC; X1000/mm³), arterial lactate (Lactate; mmol), and percent change in body weight from pre-sepsis value (% Wt.). n=5 - 15 * p<0.05 compared to pre-sepsis baseline, † p< 0.05 compared to day 3 values

i.e. number of punctures and size of needle (25). Clearly this introduces several factors which may vary between investigators. Another technical point which may result in widely disparate outcomes involves the location of the ligating suture around the cecum in the experimental animal. Altogether, these factors may not be as significant if consistently performed by the same investigator; however, these may become substantial *between* investigators as the reproduction of results may be difficult to achieve. Clearly a more standardized model of sepsis would be beneficial when these issues are considered. As a result, our lab utilizes a method of sepsis where a quantified dose of cecal contents is used as injection material into the peritoneum to induce sepsis in experimental animals.

The method of sepsis induction in rats utilized in our lab involves the intraperitoneal injection of cecal slurry (28-30). In pigs, this method was shown to be superior to intraperitoneal infusion of laboratory-grown bacteria in generating a chronic septic profile (31). A 0.25 cm vertical midline abdominal incision is made and cecal slurry is injected homogeneously throughout the peritoneum under direct vision. The incision is closed with an interrupted silk suture and the abdomen is gently massaged to distribute the injectate. Cecal slurry (200 mg cecal material/kg) is prepared by mixing cecal contents obtained from donor rats with 5% dextrose in water (D₅W) to yield a concentration of 200 mg cecal material in 5 ml. The slurry is prepared fresh for each use and is administered within 2 h to the experimental animal. Some of the characteristic changes associated with sepsis in this model are displayed in table 1. Within hours of the septic challenge, rats begin to manifest signs of septic shock such as piloerection and lethargy. Mean arterial blood pressure falls transiently, pulse pressure widens, and arterial blood lactate rises. Deaths in this model generally occur before 24 hours (~5%), and between 5 and 7 days (40%).

At 24 hours, rats are hemodynamically stable, but display tachycardia, elevated arterial blood lactate, and leukopenia. By day 3 (72 hours) after sepsis induction, mean arterial pressure [MAP - 1/3 (systolic blood pressure - diastolic blood pressure) + diastolic blood pressure] is similar to pre-sepsis values, but pulse pressure remains widened - primarily due to a significant reduction in the diastolic arterial blood pressure (data not shown). Also by day 3, lactic acidosis continues, and leukocytosis becomes evident. At 7 days after sepsis induction, MAP falls significantly, pulse pressure narrows (significantly different from pre-sepsis and day 3 of sepsis), heart rate elevates, and arterial blood lactate and white cell counts rise significantly over that seen on day 3 of sepsis.

Septic rats undergo significant weight loss by 48-72 hours, and do not regain baseline weight by day 7 (compared to pre-sepsis values); while non-septic animals gain 9.5 ± 3 % weight over the same 7 day period. Non-septic rats display no significant changes in any other of the shown parameters over 7 days after a sham surgical procedure.

We also have developed indices to identify moribund rats utilizing the hemodynamic parameters of heart rate, systolic pressure, diastolic pressure, and mean blood pressure. A reliable index of 24 hour mortality may be predicted using the following criteria: 1. pulse pressure divided by diastolic blood pressure ± 50; and 2. diastolic blood pressure ± 90 mmHg. This index has a sensitivity of 94%, selectivity of 84%, diagnostic accuracy of 90% and positive predictive value of 92% (32). The advantages of this method of sepsis induction are: (1) technically, it allows for less inter-investigator variation; and (2) lethality is less severe allowing for more accurate approximations of clinical realities. The major disadvantage lies in the requirement to sacrifice donor animals for the cecal slurry preparation.

These data indicate that this model of sepsis is associated with early changes consistent with septic shock (hypotension, elevated lactate,

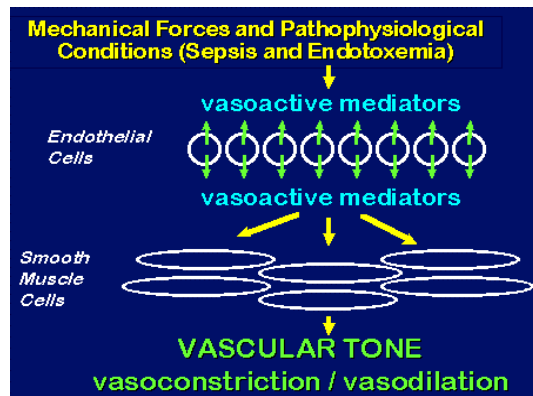


Figure 1. Mechanical forces and pathophysiological conditions (including sepsis and endotoxemia) cause activation of endothelial cells in an autocrine or endocrine manner releasing a myriad of vasoactive mediators: cytokines, endothelins (ET), nitric oxide (NO), etc. Other tissue metabolites (adenosine) act directly on smooth muscle to induce vasodilation (not shown in figure). The biological algebraic sum of these vasoactive mediators [vasoconstriction (ETs) and vasodilation (NO, adenosine)] determine vascular tone.

leukopenia), followed by a period of hyperdynamic sepsis with hemodynamic compensation - but with deteriorating conditions through day 7 of sepsis. The increasing blood lactate and leukocytosis are also consistent with clinical sepsis, and indicate an ongoing septic process. This model also allows the study of the septic condition at various stages in the course of the disease.

As a variation of the cecal slurry model, Martineau *et al.* recently described a model of rodent sepsis utilizing an infusion system which infuses a standardized *E.coli* inoculum simulating a slow leakage of bacteria into the peritoneal cavity (33). The advantage of this method lies in the standardized bacterial dose administered under controlled experimental conditions. The authors state that this model results in a reduced intergroup variability in lethality which would reduce the variability seen in model such as CLP, or cecal slurry methods. The primary disadvantage exists primarily in the elaborate system required for bacterial peritoneal infusion. Since this is a recent entry into the field of septic modeling, time and degree of utilization will determine if it will be widely accepted and become a viable option in the search for a clinically relevant model of sepsis.

4. REGULATION OF VASCULAR RESPONSES DURING THE PROGRESSION OF SEPSIS

Endothelium - formerly thought to be an inert structure - is now recognized as an

endocrine/paracrine structure which secretes several vasoactive mediators. The state of the vascular endothelium during sepsis is paramount in assessing the vascular mediation in maintaining vascular tone. The control of vascular tone is dependent on neural factors, humoral factors, mechanical forces and the physiological milieu of the endothelium (Figure 1). These factors acting either directly on vascular endothelium, or indirectly through other substances affect the diameter of the resistance vessel. To date a large number of these factors have been identified (34); however, the mechanism of vascular control during endotoxemia and sepsis is not well understood. Several studies addressing this issue have centered on the endothelium's ability to produce endothelium-derived vasoactive factors as a primary determinant of vascular fitness (35,36). Our laboratory has emphasized the influence of these neural, humoral (adrenergic), endothelial factors (nitric oxide and endothelins) and cellular metabolic products (adenosine) in the maintenance of blood perfusion during endotoxemia and more recently during sepsis. In this review, we will explore the advancements made in this area and clarify the current information regarding the underlying mechanisms responsible for sepsis-induced vascular derangements in the splanchnic circulation.

4.1 Cytokines

Cytokines are soluble polypeptides elicited in a number of pathophysiological states including inflammation, septic shock and trauma. Although traditionally known to modulate the immune system, their effects are not limited exclusively to the components of the immune system. These polypeptides are produced by a variety of cells, including monocytes, macrophages, lymphocytes, epithelial, endothelial and parenchymal cells of the gastrointestinal tract. In normal physiology, cytokines function at low levels and help to maintain homeostasis. However, after any inflammatory insult, these mediators are produced at the site of injury by a large number of cells and affect the biological system.

Even though our understanding of the host response to endotoxemia/sepsis has progressed steadily, the precise role of the individual cytokines-particularly as vascular mediators-is extremely limited. It is well known that the majority of the physiologic, metabolic, and immunologic responses to immune challenges are not due to endotoxin but rather cytokines (Figure 2). These proteins operate in concert with hormones and humoral mediators to elicit and orchestrate the cellular response to sepsis/endotoxemia. Cytokines bind to specific plasma membrane receptors resulting in gene expression which both augments and attenuates the immune response. The effect of cytokines on

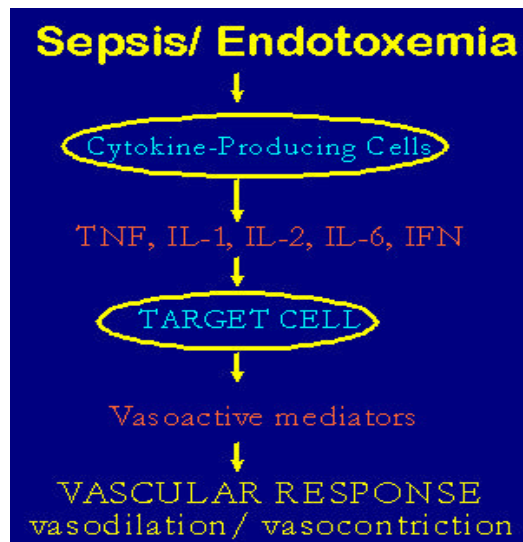


Figure 2. The cytokine signaling-response system: tumor necrosis factor (TNF), interleukins (IL), and interferons (IFN) are produced after stimulation by endotoxemia, bacteria or peritoneal sepsis. The Cytokine producing cells are: lymphocytes, macrophages, fibroblasts, etc. The effector cells are endothelial cells, hepatocytes

peripheral vascular tone is well known as hypotension often results after cytokine induction. However, direct splanchnic vascular mediation due to cytokine release has not been firmly established to date.

Though cytokines are not directly involved in controlling vascular responses (37), they are now known to induce the production of vasoactive mediators. In the following sections, we will briefly review some of the cytokines in relation to their role in splanchnic vascular control.

4.1.1 Tumor necrosis factor-alpha mediated mechanisms

Tumor necrosis factor (TNF)-alpha, produced primarily by activated macrophages and helper T-cells (38-40) is the most proximal cytokine mediator. It is detected in the serum within 20 minutes after an immune challenge (41). Its concentration peaks between 90 minutes and 2 h after endotoxin injection (42). TNF-alpha has a half-life of 14-18 minutes (43) and is degraded in the liver, skin, GI tract, and kidneys (41). Some of its actions are to increase the production of other cytokines and growth factors, release of neutrophils from bone marrow (44-47), mobilization of fat stores and amino acids in muscle (48-51) resulting in catabolic wasting, increasing the expression of intracellular adhesion molecules (ICAM)(52), and increased

production of inducible nitric oxide (53-57). Studies have shown that TNF- alpha in septic patients usually has its highest concentration upon hospital admission and may remain elevated in those patients that eventually succumb to multi-system organ failure (58). For this reason, it is advantageous to assess its role in splanchnic vascular mediation.

Recombinant TNF-alpha administration in non-septic animals has been shown to cause vascular endothelial cell dysfunction without reducing cardiac output and tissue perfusion (59,60). The administration of a TNF- alpha inhibitor prior to the onset of sepsis in the rat model has been suggested to maintain aortic ring vascular structure and function (61). Whether these findings may be extrapolated to changes prevalent in the microvasculature remains controversial.

TNF-alpha may adversely affect splanchnic perfusion by its ability to induce the expression of ICAMs on the endothelial cell surface. These molecules may impede bloodflow due to the changes that may occur in viscosity as a result of their production. Vascular endothelial cells are located between blood and tissues and are vital in the action of cytokines (52,62). ICAMs serve as "glue" for intercellular attachment. A soluble form of ICAMs (sICAM) is released into the bloodstream by monocytes, neutrophils, and endothelial cells during sepsis (63). Endotoxin and inflammatory cytokines have been shown to quantitatively increase the number of adhesion molecules on vascular endothelium and in the circulation. Significant correlation has been shown between sICAM-1 levels, TNF-alpha and endotoxin (52,62). This evidence suggests that the rheological changes in the circulation possibly induced by TNF-alpha due to ICAM production may result in an overall impairment in splanchnic perfusion by increasing the viscosity of the bloodstream.

4.1.2 Interleukin - mediated mechanisms

Shock and its sequela can be demonstrated when IL-1 is administered alone or in combination with other pro-inflammatory cytokines (64). IL-1 binds to specific cellular receptors designated as type I and type II. The Type I receptor is a transmembrane glycoprotein with a molecular ratio (Mr) of 80,000. It is a member of the Ig- protein family with three Ig-SF C2 set domains (65,66). This receptor is important in signal transduction after interleukin binding (66). The type II receptor is a glycoprotein with an Mr of 60,000, with three Ig-SF C-2 set domains in its extracellular region, a 29 amino acid cytoplasmic domain and a single transmembrane peptide segment (65,66). This receptor does not lead to signal transduction

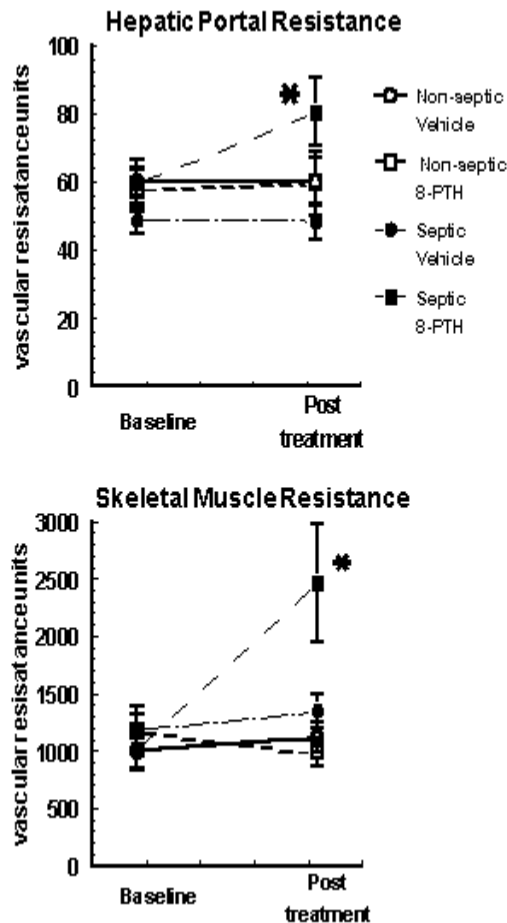


Figure 3. Hepatic portal and skeletal muscle resistance in septic and non-septic rats after treatment with 8-PTH or vehicle.

mechanisms and may serve as a decoy to reduce the amount of IL-1 available to bind the type I receptor (66,67). Since arterial and arteriolar structural integrity is vital to vascular regulation, and since endothelial derangements occurs in states of severe shock, the effects of interleukin on the vasculature is important in determining its role in splanchnic vascular control.

A novel study by Sutton *et al* Examined the structural integrity of endothelial (aortic) cells after endotoxemia in normal animals and in those genetically devoid of the type I IL-1 receptor (68). Ultrastructural comparisons revealed that the knockout mice treated with endotoxin showed total maintenance of endothelial structural integrity as compared to wild type animals receiving endotoxin (68). These findings support previous work performed by Norman *et al.* who showed that IL-1 antagonism during a lethal sepsis model in rodents exerts a protective effect by maintenance of aortic

endothelial architecture and maintenance of vascular tone (69).

One potential mechanism of endothelial damage due to IL-1 is speculated to be its ability to induce the production of IL-6. IL-6 is elevated in septic shock and has been shown to correlate with clinical outcomes in some animal studies (64). Sutton's study also monitored IL-6 levels and demonstrated a correlation between endothelial damage and IL-6 concentrations. In wild-type control animals treated with endotoxin, an 87-fold increase in the serum level of IL-6 and endothelial damage was observed. The knockout animals, which exhibited a 5-fold increase in IL-6, showed no endothelial damage (64). A subsequent increase of the endotoxin dose by 5-fold in wild-type control animals resulted in a 94-fold increase in IL-6 concentration yet without producing endothelial damage. This shows that, in this model, there appears to be no correlation between IL-6 levels and the compromise of the endothelial integrity.

4.2 Complement - mediated mechanisms

TNF-alpha alone and in synergy with endotoxin has been shown to also activate the complement pathways. Certain bacterial cell wall lipopolysaccharides directly activate the alternative pathway (38). The fifth component of C5 becomes cleaved and activated during the complement cascade giving rise to C5a. This is rapidly converted by serum carboxypeptidase N to C5a des Arginine - thought to be the mediator (or co-mediator) of the resultant systemic vascular effects. C5a, an alternative complement component and significant peripheral vascular mediator during inflammatory states, is well-known to exert a vasodilating effect on the vasculature (70). In rats, endotoxin bolus results in an increase in C5a concentration which reach baseline values after 30 minutes (70). The resultant arterial hypotension was attenuated with the administration of anti-C5a antibodies - a non-polymorphonuclear-leukocyte effector. This implicates a causal relationship. Since indomethacin inhibition abolished the hypotensive response it was hypothesized that C5a - induced hypotension occurs via a prostanoid-mediated mechanism (70).

The effect of C5a on splanchnic vasomotor regulation during sepsis or endotoxemia has, to our knowledge, not been completely characterized. Lundberg *et al* assessed regional hemodynamics in nonseptic rats after C5a challenge and observed a reversible systemic hypotensive response associated with the release of vasodilating and vasoconstricting products of the cyclooxygenase pathway (71). In addition, they reported a 25% decrease in both cardiac output and hepatic blood flow after C5a

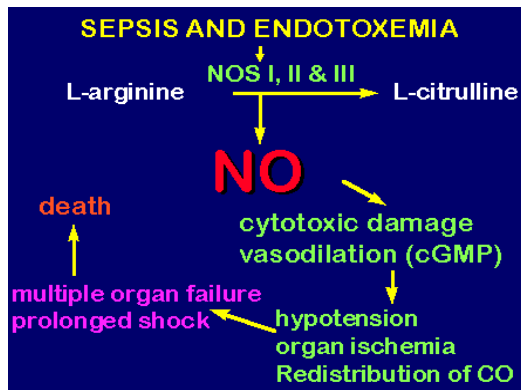


Figure 4. Synthesis and actions of nitric oxide (NO): NO synthase exists in three isoforms: NOS I & III are constitutive producers of NO. NOS II is the inducible isoform. (CO: cardiac output).

administration (71). They attributed their findings of arterial hypotension to dual mechanisms involving a thromboxane A₂ - induced vasoconstriction in the pulmonary bed resulting in decreased cardiac output and a prostaglandin I₂ - mediated peripheral vasodilation. Although extrapolation of these findings to septic models must be viewed with caution, this study suggests that some aspects of splanchnic vasoregulation is mediated through the alternative complement pathway.

4.3 Adenosine - mediated mechanisms

Adenosine is recognized as a potent vasodilator. Adenosine is produced in numerous tissues after the degradation of AMP by 5' nucleotidases (72). Ubiquitous in physiological systems, it plays an active role as a regional regulator of tissue perfusion via a receptor-mediated process which produces vasodilation in hypoxic tissues and redistributes blood within hypoxic vascular regions (73,74). In an intravenous *E.coli* septic model in rats, quantitative changes in the adenine nucleotide pools were shown in the liver and small intestine (75). Even though direct measurements of adenosine and/or its catabolites in septic tissues was not done, this has been performed in rats subjected to hemorrhagic shock and significant elevations of adenosine and catabolite products were observed (76).

The effects of adenosine on intestinal micro-circulation has been delineated by Granger and Norris (77). Using a canine model, they reported that theophylline, an adenosine receptor antagonist, significantly reduced mesenteric flow responses in chow fed animals and no effects in unfed animals. This suggests an augmented role of adenosine during an increase in metabolic activity.

Evidence from our lab has shown that adenosine is actively involved in the redistribution of blood flow toward the splanchnic organs and skeletal muscle during sepsis and during an increased metabolic activity (78). We have shown that blockade of adenosine receptors in rats during sepsis, utilizing the non-selective adenosine receptor antagonist, 8-phenyl-theophylline (8-PTH), resulted in an increase in hepatic-portal and skeletal muscle vascular resistance (Figure 3) as compared to non-septic controls (78). In addition, an organ-specific effect was seen in septic and nonseptic animals treated with 8-PTH. When compared to its vehicle, 8-PTH caused a significant decrease in flow to small intestine, cecum, colon, and pancreas in septic rats which were not seen in non-septic controls.

These results imply that there is an increased production of adenosine during the septic state which can be attributed to two pathways: (1) a reduction in the tissue oxygen tension due to either increased oxygen supply or increased consumption; or (2) increased adenylyl cyclase activity resulting in increased adenosine production from cAMP degradation providing substrate for adenosine formation. Alterations in oxygen supply-demand dynamics is critical in the pathogenesis of sepsis. Adenosine, on-the-other- hand, an important energy metabolite, is an important modulator of perfusion to the hepato-splanchnic circulation during sepsis.

4.4 Nitric oxide - mediated mechanisms

Nitric Oxide (NO), a potent vasodilator, is produced by vascular endothelial cells from its precursor L-arginine which is subsequently converted to citrulline by nitric oxide synthase ENOS (Figure 4). NO, by virtue of affecting vascular smooth muscle guanylate cyclase mediates the vasodilating effect. NOS exists in three isoforms characterized by their pattern of activity (i.e. constitutive vs. inducible) and their requirement for calcium. Types I and III are located in the nervous system and endothelium respectively and are classified as constitutive, calcium dependent isoforms. Type II is located primarily in macrophages and is classified as an inducible, calcium independent isoform. Type II NOS is stimulated by septic mediators such as endotoxin and cytokine (79) and requires 4-6 hours for expression after an immune challenge (80). Due to its association with calmodulin, quantitatively more NO is produced from this isoform than the other two (nanomoles vs. picomoles) (81).

Numerous studies have attempted to inhibit nitric oxide production by NOS during sepsis. Most have reversed sepsis-induced hypotension yet uniformly resulted in poor mortality outcomes (82-88). Rubin *et. al.* harvested aortic endothelial cells from guinea pigs 16 h after induction of

endotoxemia. The production of NO, in a concentration-dependent manner, decreased after exposure to endothelium receptor-dependent agonists and not endothelium receptor-independent agonists (89). Thus, the use of NOS inhibitors during this time period in sepsis may further compromise NO production leading to vascular tone derangements and compromised tissue perfusion (89-91).

Inhibition of cNOS by L-NMMA (nitro-guanido-monomethyl-L-arginine) reduces splanchnic perfusion and exacerbates intestinal vascular injury induced by endotoxin challenge (92,93). More recently, the focus of attention has shifted to the inhibition of iNOS since this isoform is selectively increased during sepsis and, quantitatively, has a greater significance (94). Several compounds have been reported to inhibit iNOS, however, their selectivity remains controversial. Data from this lab assessed the ability of aminoguanidine in attenuating NO production after chronic sepsis in rats. After 30 minutes of infusion, aminoguanidine led to a significant decrease (65 micromolar vs. 90 micromolar) in the serum nitrates. The serum level of nitrates returned to previous septic levels once infusion was terminated (95). Further studies in our lab have elucidated an important role of the inducible form of NOS in regulation of perfusion during sepsis. Administration of aminoguanidine resulted in significant decreases in perfusion to ileum in septic rats compared to controls (unpublished data).

It also has been known that septic individuals and endotoxemic or septic animals demonstrate impaired responsiveness to sympathetic nerve stimulation and to exogenously administered adrenergic agonists. Recent evidence has implicated NO as a main factor in the generation of this vascular unresponsiveness (96). Since it has been shown that vascular responses to calcium were affected during sepsis, it is thought that a defect in contractile machinery is responsible for some of the unresponsiveness and to the hypotension during sepsis. Ex vivo aortic preparations have displayed endothelium-independent responsiveness to norepinephrine which is restored by the addition of NOS inhibitors (96) implicating NO as an important contributor to septic-induced vascular derangements. Additional evidence for the role of NO stems from estimation of cGMP demonstrating a significant elevation in vessels removed from endotoxemic rats as compared to controls (97).

In vivo studies in endotoxemic rats assessing pressor responses to norepinephrine resulted in reductions of responsiveness as assessed by changes in the arterial blood pressure. These alterations were restored to normal levels following the administration of the NOS inhibitor L-NMMA

(96). This evidence from *In vivo* and *ex vivo* studies suggest that NO plays an important role in changes in the vascular tone during sepsis which may be inferred to also occur at the splanchnic vasculature.

Hemoglobin is a well-recognized scavenger of nitric oxide. Its actions are in part through a NO-mediated pathway independent of NO synthesis. A recently developed and modified hemoglobin based blood substitute, diaspirin cross-linked hemoglobin solution (DCLHb) has been shown to interact closely with NO (98) and Endothelins (ET) (99,100), potent vasoconstrictors. DCLHb, a stroma free, non-antigenic, oxygen-carrying molecule, has been shown to improve regional blood flow in the treatment of hemorrhagic shock (101-103). DCLHb has pressor qualities independent of alpha-adrenergic pathways (104-107).

In our laboratories, studies were performed with the hypothesis that DCLHb infusion would improve organ perfusion in cecal-slurry induced septic rats. Rats received either 100 or 250 mg/kg DCLHb or albumin at 1, 2, or 4 h after induction of sepsis. Moribund rats were identified and received 100 mg/kg DCLHb or iso-oncotic albumin infusions at identical time points. Radiolabeled isotopes were infused pre-sepsis induction, pre-DCLHb infusion and post-DCLHb infusion to assess changes in tissue blood flow. In rats that received DCLHb, all regions of the GI tract demonstrated significant increases in perfusion 24 h after sepsis induction (32). Perfusion to the kidneys, pancreas, and liver were not significantly altered. This particular study also showed an increase in systemic vascular resistance after treatment but not at the expense of specific organ system perfusion - the ultimate goal of resuscitation therapy.

Anecdotally, DCLHb treated rats displayed reduced peritoneal extravasation at necropsy compared to albumin treated rats. Cecal contents also were observed to appear unusually dry. Together, this may indicate that modified hemoglobin (DCLHb) may provide protection to intestinal vascular beds and prevent the loss of vascular fluid to the third space compartments in the form of ascites. However, further studies are still needed to confirm that this protective effect of hemoglobin preparation is due to its interactive role with NO and or other (i.e. endothelin) mechanisms.

4.5 Endothelin - mediated mechanisms

Endothelins (ET), potent vasoconstrictors also produced by vascular endothelium, have recently gained acceptance as important mediators of vascular tone (108), particularly in sepsis. They are 21-amino

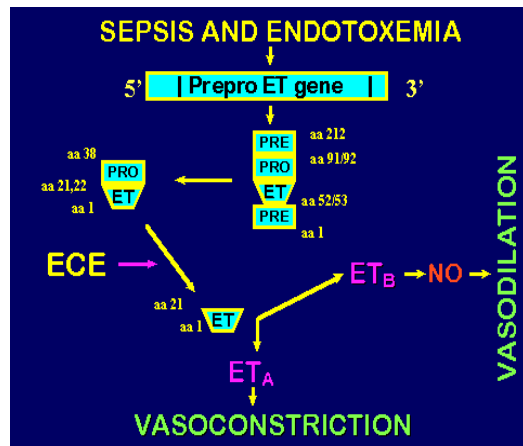


Figure 5. Role of endothelin pathways in sepsis and endotoxemia. aa=amino acid, ECE=endothelin converting enzyme, proET (big endothelin)

acid peptides consisting of ET-1, ET-2, ET-3, and vasoactive intestinal contractor (VIC). ET-1 gene expression is up regulated by endotoxin or LPS in human macrophages (109). Cytokines have also been shown to affect ET gene expression (109).

Produced from the cleavage of 203-213 amino-acid proteins called preproendothelins into precursors called big endothelins (big ET-1, big ET-2, big ET-3), their final conversion is dependent on metallo-endoproteases called endothelial converting enzymes (Figure 5) generating ET-1, ET-2, or ET-3. Three isoenzymes (ECE-1a, ECE-1b, and ECE-2) have been isolated to date. ET's binding directly to their specific receptor results in alterations in vascular tone. These G-protein-coupled receptors - ET_A, ET_B, and ET_C - elicit responses that are dependent upon the concentration ratios of ET's: ET_A receptors are activated when ET-1 is equipotent to ET-2 but more potent than ET-2 (1=2>3); ET_B receptors are activated when ET-1=ET-2=ET-3 (1=2=3); ET_C receptors are activated when ET-3>ET-1 or ET-2 (3>1,2) (110-112).

As described above, besides ET's own independent effects on vascular tone, ET production is keenly interrelated with other mediators during sepsis. An exogenous challenge of ET induces the release of prostanoids (thromboxane A₂ and prostacyclin) and nitric oxide (113) among others (114). Additionally, cytokines (TNF- α , IL-1 α , IL-1 β , IL-2 or IL-6) also cause increased production of ET and release from vascular endothelial cells. Even though stimulants for ET production are most likely multi-factorial, TNF- α appears to be required for its release. Administration of anti-TNF antibodies prior to TNF- α infusion in rats (115) or LPS treatment in pigs (116) significantly reduced circulating ET-1 levels.

The role of ET in sepsis has been implicated by several observations: Plasma ET concentrations are elevated in septic patients (117); plasma ET levels double during sepsis in rats (118); ET-1 anti-serum improves hemodynamics and alleviates shock injury in septic animals (119). These studies demonstrate the potential of ET-mediated mechanisms in the pathophysiology of sepsis. The profile of ET production at various time points during sepsis and whether varied release of ET alters the progression of sepsis is unknown.

In a recent study, we hypothesized that induction of intraperitoneal sepsis would not only alter myocardial performance but also the circulating and myocardial concentrations of ET and NO during sepsis. This study was undertaken to determine 1) myocardial performance at 24 and 48 hours following sepsis/sham sepsis, and 2) the profile for ET and NO in the plasma and left ventricular tissue during sepsis. The results indicated that progression of sepsis in our rat model may occur at least in two phases. Phase 1 (early phase, 0-12 h after induction of sepsis), when both plasma ET and NO showed an increase in response to induction of sepsis and phase 2 (late phase, 12-48 h after induction of sepsis), when plasma ET levels returns to basal levels while NO remained elevated. Thus, these two potent vasoactive agents have a divergent time course that is likely to be related to the different mechanisms of control of the vascular tone during sepsis (120). The implication of this observation on the regulation of splanchnic perfusion has not been assessed directly, however, it is likely that ET-mediated mechanisms play a significant role in splanchnic vasomediation during sepsis.

5. HEPATOSPLANCHNIC PERFUSION DURING ENDOTOXEMIA

Several investigators have hypothesized that sepsis results in the ischemia of GI mucosa causing translocation of microorganisms and endotoxin into the peripheral circulation through the portal circulation (121). Translocation is the passage of viable bacteria from the lumen of the GI tract into other organs systems via the mesenteric lymphatic system (122-125). The exact mechanisms surrounding translocation remain controversial. Endotoxemia results in a decrease in splanchnic organ perfusion (16,126) while the response to sepsis is variable among organs and their tissues (28,29). Traditional views have pointed towards an increase in intestinal permeability due to endotoxin-induced changes in mucosal permeability; and evidence exists to support this notion (125,127). Many have attributed this increased permeability after endotoxin challenge to a splanchnic mucosal hypoperfusion with hypoxia and acidosis (128,129). In contrast, Fink *et al* (130)

Table 2. Comparison of the effects of naloxone to naloxone MB in endotoxic shock

	TIME	SALINE	NALOXONE	NALOXONE MB
Mean arterial blood pressure (mmhg)	0	120 ± 7	102 ± 6	122 ± 4
	30	121 ± 5	100 ± 5	123 ± 5
	60	89 ± 5*	95 ± 6	103 ± 4 ^l
	120	104 ± 4	89 ± 5	103 ± 3 ^l
Cardiac output (ml/min)	0	136 ± 10	125 ± 11	115 ± 9
	30	106 ± 9	107 ± 19	107 ± 16
	60	59 ± 9 ^l	87 ± 9	66 ± 8 ^l
	120	59 ± 9 ^l	58 ± 9 ^l	72 ± 8 ^l
Small bowel blood flow (ml/minxg tissue)	0	1.21 ± 0.14	1.62 ± 0.18	1.53 ± 0.53
	30	1.02 ± 0.28	1.54 ± 0.28	0.98 ± 0.22
	60	0.52 ± 0.06 ^l	1.38 ± 0.32 ²	1.12 ± 0.28
	120	0.37 ± 0.10 ^l	1.03 ± 0.32 ^{l,2}	0.83 ± 0.09 ^{l,2}
Adipose tissue blood flow (ml/minxg tissue)	0	0.25 ± 0.06	0.30 ± 0.07	0.25 ± 0.03
	30	0.17 ± 0.04	0.20 ± 0.08	0.13 ± 0.02
	60	0.06 ± 0.03 ^l	0.15 ± 0.09	0.05 ± 0.03 ^l
	120	0.03 ± 0.01 ^l	0.04 ± 0.01 ^l	0.06 ± 0.01 ^l
Renal blood flow (ml/minxg tissue)	0	6.64 ± 2.30	4.46 ± 1.44	4.99 ± 1.63
	30	5.32 ± 1.72	5.48 ± 1.95	4.40 ± 0.70
	60	2.24 ± 1.07 ^l	2.51 ± 1.01 ^l	2.26 ± 0.92 ^l
	120	1.37 ± .037 ^l	1.82 ± 0.41 ^l	1.02 ± 0.27 ^l

^l $p \leq 0.05$ compared to $t=0$

* $p < 0.05$ compared to saline treated value

provided evidence, in a porcine model of normodynamic septic shock, that an increase in mucosal oxygenation may occur and that mucosal hypoxia is not responsible for the development of mucosal acidosis (131). This subject remains in dispute and further studies are required to further elucidate the exact mechanisms involved.

5.1 Opiate antagonism during endotoxemia

Endogenous opioids are released during sepsis and septic shock and contribute to the loss of vascular tone. Additionally, exogenously administered opioids alter perfusion patterns seen in nonseptic states (132). Naloxone, an opioid receptor antagonist, has been shown to reverse the hypotension which is often associated with endotoxemic shock (133-136). This manifests as an increase in the mean arterial pressure and/or systemic vascular resistance. Previous studies have shown that naloxone results in improved hemodynamic parameters after the institution of the shock state. Increased peripheral resistance has been demonstrated after naloxone treatment of hemorrhagic shock in porcine models (137). Schadt and York showed that administration of naloxone reversed tachycardia in hemorrhaged rabbits (138).

Although seemingly beneficial, the advantage of the increased systemic blood pressure resulting from increases in systemic vascular resistance are dependent upon which organs undergo increases in resistance. Since endogenous catecholamines are important to the effects of naloxone (139-141), and since the actions of naloxone have different effects on different vascular beds, the above findings gave no insight into the perfusion state of vital organs. For this reason, work from this lab has further elucidated the effects of naloxone on hepato-splanchnic perfusion and its mechanism of action.

The effects of naloxone on regional - particularly hepato-splanchnic - perfusion has been studied extensively in this laboratory. In early studies, conscious, awake rats were given an endotoxemic challenge prior to treatment with naloxone. Naloxone did not uniformly affect regional vascular resistance. However, the hepato-splanchnic region showed an increased perfusion as evidenced by an increased hepatic portal blood flow at 10 and 30 minutes post-endotoxin infusion. Hemodynamic studies revealed a significant increase in the systemic blood pressure after infusion of naloxone in endotoxemic animals. Cardiac output, decreased after endotoxemia alone, showed no appreciable change after infusion of naloxone in endotoxemic rats. This work established beneficial effects of opioids during endotoxic shock, showing its ability to achieve improvements in blood pressure and cardiac output.

5.1.1 Naloxone: are the perfusion effects centrally mediated?

Since opioid receptors are located in the central nervous system as well as on the peripheral vasculature, studies from this lab focused on determining the site of action that accounts of the improvement of hepato-splanchnic perfusion during endotoxic shock. In a recent study (142), we used naloxone methyl-bromide (NMB), a NLX derivative which does not cross the blood-brain barrier, and compared its effects on perfusion to that of NLX during endotoxin shock. Selected data are shown in table 2. Consistent with our previous reports (134,135), after administration of endotoxin, NLX prevented the 60 minute decline in cardiac output, and improved perfusion in small bowel and other regions of the GI tract (effect on other regions not shown), and epididymal fat pads (adipose), but had no significant effect on the depressed renal perfusion. NMB did not prevent the decline in cardiac output, nor change perfusion of adipose tissue, suggesting that these effects were mediated via central neural mechanisms. However, the improvement in small bowel and other GI blood flows were still seen, indicating that these responses were mediated via actions at peripheral opioid receptors.

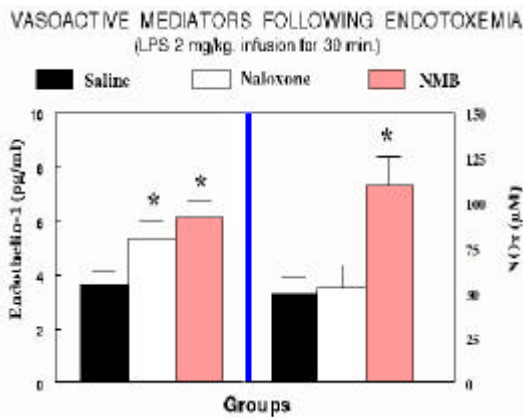


Figure 6. Effect of naloxone and naloxone methyl bromide on the concentrations of endothelin-1 (left panel) and nitric oxide (right panel) in blood plasma following administration of LPS in rats.

5.2 ET and NO interactions

To explore the influence of endotoxemia and opiate antagonism (central or peripheral) during endotoxemia on vasomediation, the concentrations of ET and NO were estimated (unpublished data; figure 6). Endotoxemia produced an increase in the ET-1 levels but not of NO at two hours after infusion of LPS (2 mg/kg per 30 min.), when compared with non-endotoxemic and non-surgically manipulated rats. Administration of naloxone, in endotoxemic rats, significantly increased ET levels, but did not affect NO. In contrast to naloxone, naloxone methobromide -the peripheral opiate antagonist - significantly increased the blood level of ET and NO, in the endotoxemic rats. The importance of these observations is multi-factorial.

These data indicate that peripheral opiate antagonism via naloxone methobromide, may activate the NO-mediated pathways to a greater extent than the non-selective opiate antagonism via naloxone. Further, both opiate antagonists cause an increase in the blood levels of ET and with significant increases of NO observed only in the naloxone methobromide group. It appears that peripheral opiate antagonism produces a more profound effect on the synthesis of ET and NO. However, we did not observe similar changes in the hepatosplanchnic blood flow in the regions studied which may be due to their physiological antagonistic effects. It is likely that peripheral opiate antagonism interacts with vasoactive mediators (ET and /or NO) for alterations of vascular tone during endotoxemia. These observations provide additional evidence for the importance of ET and NO during endotoxemia. However additional work is required to better

elucidate its exact physiological implications and importance.

5.3 Influence of adrenergic mechanisms.

Despite elucidation of the potential beneficial effects of naloxone - induced opioid blockade during endotoxemia, the physiological mechanisms involved remained unclear. Opioids may act to attenuate the action of catecholamines (135). Since catecholamine levels are persistently elevated in human septic shock (143), adrenergic enhancement has been proposed as a possible mechanism by which the effects of naloxone were mediated.

This was alluded to in the work of Allgood *et al* (144) who showed that adrenal demedulation and ganglionic blockade reduces the effectiveness of naloxone during endotoxic shock. Malcolm *et al* demonstrated that naloxone could enhance the effects of an exogenously administered epinephrine during endotoxin shock (145). Although important, these studies did not address the regional perfusion changes. As a result, studies by Law *et al.* set out to determine the role of beta-adrenergic activity involved in the action of naloxone - especially related to the regional perfusion of various organs (133). These studies revealed that the opioid-antagonism induced improvements in regional perfusion during endotoxic shock. This effect was ablated in the presence of the beta-adrenergic receptor blocker, propranolol, in the gastric, colonic, and splenic regions. However, naloxone-induced improvement in small intestinal perfusion during endotoxic shock was not affected by beta-adrenergic receptor blockade (133).

Hemodynamically, mean arterial blood pressure did not significantly decrease during or after endotoxin administration. This was expected as the dose used in this study (4mg/kg + 2 mg/kg*hr) was less than the study previous described (10 mg/kg). These rats, however, developed a tachycardic response which was ablated by both naloxone and propranolol. Endotoxemia resulted in a significant decrease in the cardiac index which was reversed with naloxone infusion. In the presence of beta-adrenergic blockade, the naloxone-induced improvements in cardiac index were prevented. Together, these results suggest a variably mediated beta-adrenergic hepato-splanchnic perfusion response to naloxone during endotoxic shock and a partial mediation of the effects of opioid antagonism on beta-adrenergic mechanisms.

Further studies from this lab determined the role of alpha-adrenergic mechanisms of naloxone mediating changes in regional perfusion during endotoxin shock (135). Prior to administration of naloxone during endotoxic shock, alpha-1 or alpha-2

receptor blockade was achieved with phentolamine or yohimbine, respectively. The response of individual organs in the hepato-splanchnic circulation to alpha-adrenergic blockade was heterogeneous. Alpha-2 receptor blockade with yohimbine ablated the effects of naloxone in both small intestine and spleen suggesting at least a partial alpha-adrenergic-mediated effect elicited by naloxone during endotoxin shock. Alpha-1 receptor blockade with phentolamine also resulted in the ablation of the effects of naloxone in similar regions. Hemodynamic changes showed a blockade of naloxone's ability to increase the mean arterial pressure and cardiac output with treatment with phentolamine but permitted an increase in systemic vascular resistance. In the presence of yohimbine, naloxone increased mean arterial pressure but not cardiac output or systemic vascular resistance.

Results from this study revealed that some of the effects of naloxone on hepato-splanchnic perfusion are mediated via its alpha-adrenergic actions. In comparing these results to those achieved after beta-adrenergic blockade, particular attention should be given to small intestinal perfusion which, after alpha-adrenergic blockade, shows an ablation of the effect of naloxone on perfusion which was not observed after beta-adrenergic blockade. This suggests that during endotoxemia the effect of naloxone on perfusion of small bowel is mediated, at least partially, through an alpha-adrenergic mechanism and exclusive of a beta-adrenergic mechanism. From a hemodynamic standpoint, this work revealed that during endotoxic shock, some cardiovascular effects of endogenous opioids are alpha-adrenergic independent.

6. CLINICAL INTERVENTIONS DURING SEPSIS

In many intensive care units, the "systemic inflammatory response syndrome" and its progression to multi-system organ failure and the role thought to be played by the gut in this occurrence has led to certain "goal-directed" therapies. Although controversial, some have suggested that patient survival is improved if certain hemodynamic parameters are maintained at supranormal levels in order to maximize oxygen delivery and in the hope of increasing oxygen consumption. However, both the GI tract and the liver may be inadequately perfused despite the presence of normal systemic measures for ensuring adequacy of tissue oxygenation (146,147). In addition, often, there is a dissociation between changes in intramucosal pH and the changes in the global measurements induced by inotropes (148) attributed to alpha-adrenergic stimulation. A continuation of inotropy to increase oxygen delivery in this setting could possibly be deleterious.

6.1 Measurement of splanchnic perfusion

Volume resuscitation to restore normal preload and baseline hemodynamic parameters does not unequivocally insure adequate organ perfusion - especially to the splanchnic beds (121). A method which allows a determination of improvements in splanchnic circulation clinically may be an important tool in the assessment and treatment of septic patients. This has been achieved by the development and acceptance of gastric tonometry. Gastric tonometry has been widely accepted since its description in 1989 (149). It has enabled the detection of splanchnic ischemia in patients who are apparently adequately resuscitated. This advancement has been based on the reasoning that when oxygen consumption is supply-dependent, the rate of ATP hydrolysis exceeds the rate of synthesis leading to a net production of hydrogen ions. Intracellular acidosis is a consequence of a decrement on oxygen delivery that is significant enough to result in supply dependency of oxygen consumption (130). A tonometer is positioned nasogastrically into the lumen of the stomach. 0.9% saline solution is infused into the silicon balloon and is allowed to equilibrate with the PCO₂ of the gastric lumen. This sample is then analyzed using a standard arterial blood gas analyzer. The pH of the mucosa is calculated using the Henderson-Hasselbach equation (130).

Another method for assessing the splanchnic perfusion has been recently compared to tonometry by Oldner *et al* (150). Microdialysis allows endogenous substances to be collected from extracellular fluid (ECF) without liquid extraction (151,152). A semipermeable tubular dialysis membrane is placed into the desired tissue and perfused with a fluid that will permit equilibration with the ECF by diffusion. The dialysate is then analyzed for metabolites of interest. With tonometry of the small intestine serving as a reference, microdialysis probes were positioned in the liver, ileum and a peripheral arterial vessel. Experimental animals (porcine) received an *E.coli* LPS endotoxin challenge that resulted in significant changes in intestinal lactate and hypoxanthine and liver lactate after three hours as compared to controls. Simultaneous tonometry revealed significant decreases in pH corresponding to tissue accumulation of lactate. Most importantly, these changes were detected prior to the detection of alterations in the peripheral blood (2.5 hr vs. 4 hr) indicating that these methods may be more sensitive to assess the adequacy of gut perfusion. This study demonstrates an advantage of the regional organ monitoring in the course of endotoxic shock. Both microdialysis and tonometry may be used as sensitive tools for assessing splanchnic metabolic changes in this setting.

The two other tests that have been used to assess splanchnic perfusion in clinical experimentation are indocyanine green (ICG) clearance and the monoethylglycinexylidide (MEGX) test. After a venous bolus, impaired ICG clearance has been shown to reflect abnormal liver perfusion and function (153). The MEGX test is a dynamic assessment of hepatic blood flow and function by measuring hepatic clearance of lidocaine (by its conversion to MEGX).

6.2 Dopexamine

Dopexamine hydrochloride was developed as an analogue of dopamine which served as a peripheral arterial vasodilator - retaining the renal vasodilating effects of dopamine without an α -adrenergic receptor agonism (154).

Systemic vasodilation is produced by stimulation of β_2 -receptors, peripheral dopamine-1 and presynaptic dopamine-2 receptors (154-156). A weakly positive inotropic activity has been postulated to be mediated by cardiac β_2 receptors or by indirect β_1 receptor activity from inhibition of neuronal catecholamine uptake (157). Its current use (not in the US) is for the treatment of heart failure and low cardiac output states following cardiac surgery. Its effects on splanchnic perfusion were assessed using radiolabeled microspheres in the anesthetized dog showing an increase in blood flow to the GI tract but not to the liver (158).

Cain *et al* Studied the effect of dopexamine in endotoxic dogs and showed that perfusion in an isolated ileum model was not increased yet lactate production decreased dramatically (159). Since lactate production by the gut may be due to oxygen supply/demand mismatches in the mucosal regions, dopexamine, in this scenario may have resulted in improved oxygenation without an increase in blood flow. This may occur if blood flow is redistributed with the mucosa being favored over the muscularis (160). Since this effect has been shown to occur with isoprenaline (161), a possible β_2 effect is implicated. The mucosa is an important site of oxygen consumption; therefore, an increase in perfusion through the mucosa should reduce anaerobic metabolism, lactate production, and A-V oxygen shunting (162). This may explain the findings in this study of significant intramucosal pH improvement without an increase in the blood flow of the bowel (160).

A second prospective randomized study performed by Smithies *et al* in 11 septic patients gastric tonometry and ICG clearance pre- and post-infusion of dopexamine were performed. The dopexamine infusion rate was progressively increased from 2-6 microgram/kg/min (163). A significant

increase in gastric intramucosal pH (compared to pre-infusion pH), and a significantly increased ICG clearance 1 hr post infusion was observed these findings correlated with an increase in cardiac index. This suggests that effects of dopamine intramucosal pH are most likely mediated via D-1 dopaminergic agonism with changes in hepatic perfusion primarily attributable to increases in cardiac performance.

6.3 Dopamine

Peripheral dopamine receptors are classified into dopamine-1 and dopamine-2 receptors. It is believed that the effect of this drug on splanchnic tissues is mediated by its dopamine-1 receptor agonism action. A single-blinded randomization study by Maynard *et al.* assessed the effect of dopamine (n=10) and dopexamine (n=10; control=5) in critically-ill patients (160). Changes in gastric intramucosal pH, lidocaine metabolism to MEGX, and ICG clearance were studied pre- and post- drug infusion. Low-dose dopexamine (1 μ g/kg/min) produced a significant change in all these measurements of mucosal perfusion without any systemic hemodynamic changes. On the other hand, dopamine had no effect on any of the measured variables.

A recent study has focused on the ability of dopamine to augment splanchnic flow in patients mechanically ventilated with positive end-expiratory pressure (PEEP) (161). The importance of this study lies in the fact that a large proportion of patients who may be served by a splanchnic vasodilator require PEEP-assisted oxygenation. The advantageous increase in oxygenation is tempered by a reduction in cardiac output due to an increase in intrathoracic pressure. This often results in decreased venous return to the right heart, and at extreme levels of PEEP, a shift of the interventricular septum resulting in a compromised stroke volume. It was hypothesized that in translocation-induced sepsis (164,165), the increased splanchnic flow response is associated with dopaminergic agonism. To test this experimentally, *in vivo* videomicroscopy to measure mesenteric A1, A2, and A3 arteriolar intraluminal radii and A1 arteriolar optical doppler velocities were measured in rats exposed to either no PEEP (control) or increasing levels (0,10,15,20 cm H₂O) of PEEP. The effect of PEEP on mesenteric blood flow (MBF) and cardiac output during the infusion of dopamine (2.5 or 12.5 μ g/kg/min) with normal saline boluses was also determined. Their results showed reduction in MBF (78%, $p<.05$) and CO (31%, $p<.05$) from baseline at 20 Cm H₂O PEEP. Dopamine infusion at 2.5 μ g/kg/min partially ameliorated both CO and MBF changes due to PEEP, yet required a normal saline bolus (4 ml) to reach baseline levels. Dopamine infusion at 12.5 μ g/kg/min with saline bolus restored CO to baseline but MBF remained 46% below

baseline levels. From this study, one may conclude that in the rat model, dopamine may serve as an adjunct to adequate fluid resuscitation to improve mesenteric perfusion. Extrapolation to clinical situations must be viewed with caution. A similar study to assess the effect of dopexamine in comparable clinical conditions has not yet been performed. Such a study is likely to generate particularly useful information since the majority of patients in the intensive care unit receive positive pressure ventilation due to underlying sepsis-induced pulmonary compromise.

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