# The Vascular Bed during Critical Illness: Evaluation in Animal Models

J. Gorrasi, J. Takala, and S.M. Jakob

## Introduction

Vascular reactivity has a fundamental role in regulating blood flow and tissue oxygen consumption. Vascular tone is regulated by receptors in endothelial and smooth muscle cells which can be stimulated by biochemical signals or a physical stimulus [1]. Receptor abundance and their response to stimuli is different among the different vascular beds, which enables fine tuning between organ perfusion and oxygen consumption according to different metabolic needs [1]. Vascular reactivity contributes to maintain the adequacy of tissue perfusion in response to acute injury such as sepsis and trauma [2]. This compensatory response can redirect regional blood flow towards organs where a decrease in oxygen consumption would have detrimental consequences for the organism such as the brain and the coronary arteries [3].

## Definition of Vascular Reactivity

Vascular smooth muscle is functionally different from striated cardiac or skeletal muscle. Vascular smooth muscle undergoes slow tonic contractions to maintain pressure and to reduce vessel diameter [1]. Actin, myosin, and troponin-like regulatory proteins are used for regulation of vascular smooth muscle contraction [4, 5]. Vascular smooth muscle contraction can be induced with electrical stimuli, stretching, and with chemical stimuli such as norepinephrine, angiotensin II, vasopressin, endothelin-1 and thromboxane A2 [1]. Each of these mediators binds to receptors that trigger metabolic pathways to increase the intracellular calcium concentration [6]. The intracellular calcium concentration is regulated by several mechanisms: Phosphoinositolphosphate and diacylglycerol increase calcium concentration in response to stimulation by norepinephrine, angiotensin II and endothelin-1; nitric oxide (NO) and cyclic guanosine monophosphate (cGMP) inhibit calcium entry from the extracellular space and, hence, contraction; $\beta$-agonists, by acting via G protein-coupled pathway increase cyclic adenosine monophosphate (cAMP) which in turn inhibits myosin light chain kinase.

Vascular reactivity can be defined as the normal response of a vessel after a chemical, physical or electrical stimulus under controlled conditions hence vascular reactivity dysfunction is characterized by a vessel response that is significantly different from a 'normal' response during controlled conditions.

## Methods for the Evaluation of Vascular Reactivity

*In vivo* vascular reactivity can be assessed by measuring the regional blood flow response to a physiological or pharmacological challenge [7]. *In vitro* vascular reactivity can be evaluated by using wire myography [8]: Isolated strips from arteries are fixed in an organ chamber, and the effects of stimulation (contraction or relaxation) are measured (Fig. 1). In the organ chamber, the biological preparation is immersed in physiological buffer and rinsed several times to wash out mediators and pharmacological agents that may have been tested before. Alternatively, vessels can be cannulated, mounted on glass pipettes and perfused at fixed flow rates or at constant pressures. Pressure changes across the cannulated vessels in response to a stimulus can then be measured [8]. Vascular reactivity of small arteries can also be evaluated by intravital (and *ex vivo*) microscopy [9]. Some of the key findings from experimental models investigating vascular reactivity are summarized in Figure 2.

## Methods for the Evaluation of Endothelial Function

The endothelium can be evaluated by the acetylcholine response test: Acetylcholine releases NO which induces vasodilatation by stimulation of smooth muscle guanylate cyclase and consequent production of cGMP [10]. Acetylcholine acts on endothelial cells by activation of muscarinic receptors and NO release from the constitutive NO synthase (cNOS). Hence, vasodilatation after acetylcholine stimulation demonstrates intact endothelial cell-smooth muscle cell coupling and endothelial function [11]. On the other hand, $N^G$-methyl-L-arginine causes endothelium-dependent contraction and inhibition of cyclic GMP formation [12].

## Mediators of Vascular Reactivity

Effects of important mediators are summarized in Table 1.

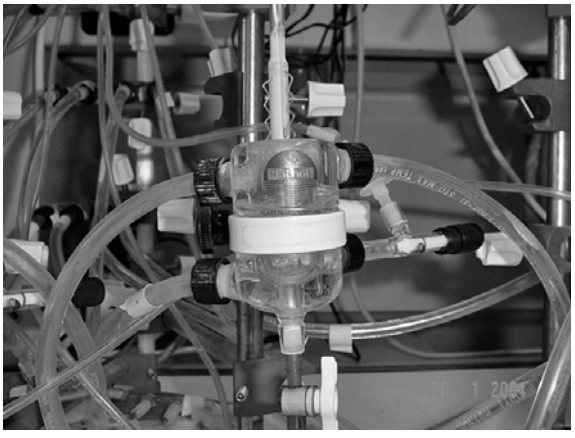

**Fig. 1.** Single organ chamber. Vascular rings are mounted in the chamber and connected to a pressure transducer. Each chamber has controlled conditions for temperature, oxygen tension and pH

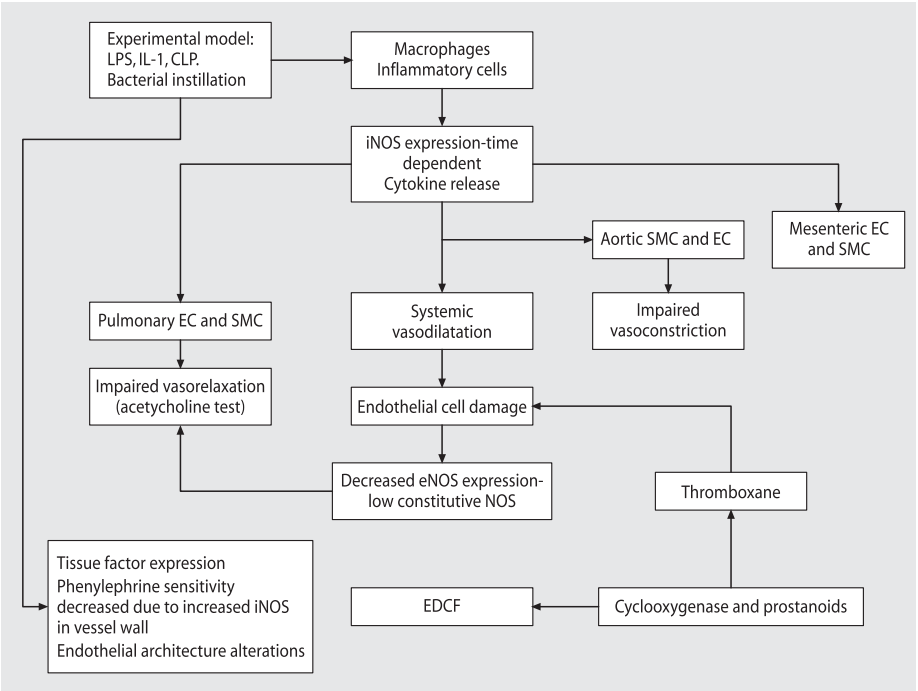


**Fig. 2.** Findings from experimental models investigating vascular reactivity. Inflammatory stimuli can induce inflammatory gene expression in macrophages and inflammatory cells with high expression of inducible nitric oxide synthase (iNOS) and cytokine proteins. These inflammatory proteins induce the release of vasoactive substances into the circulation. As a result, systemic vasodilatation (*in vivo*) and impaired vasoconstriction in response to adrenergic compounds (*in vitro*) occur. At the same time, these inflammatory mediators induce endothelial dysfunction and cell damage. LPS: lipopolysaccharide; IL-1: interleukin-1; CLP: cecal ligation and puncture; SMC: smooth muscle cells; EC: endothelial cell; eNOS: endothelial nitric oxide synthase; EDCF: endothelial derived contracting factor

## Adenosine

The effect of adenosine on arteries has been assessed in an isolated dual-perfused liver model [13]. ATP administration elicited hepatic arterial vasodilatation. Furthermore, the administration of a purino-receptor antagonist inhibited the response to injected ATP. In ischemic conditions, ATP is metabolized to adenine nucleotides, such as adenosine in order to provide energy. Adenosine mediates increases in local blood flow at different vascular beds: It has been reported that adenosine dilates coronary arteries to allow matching between oxygen supply and consumption [14].

## Prostanoids

It has been postulated that attenuated pulmonary and systemic vascular contractility is – at least in part – mediated by prostanoids and prostaglandins. This was tested in a rat model of hyperdynamic sepsis induced by cecal ligation and perfora-

**Table 1.** Effects of drugs commonly used to test vascular reactivity

| Substance | Smooth vascular cell | Endothelial cell |
|---|---|---|
| Norepinephrine | Contraction | |
| Phenylephrine | Contraction | |
| KCl | Contraction | |
| Acetylcholine | Relaxation, at high doses contraction | Activation of muscarinic receptors |
| ACE inhibitors | | NADPH oxidase modulation |
| Sodium nitroprusside | Relaxation | |
| Adenosine | Relaxation | |
| Interleukin-1 | Relaxation | |
| Thromboxane | Contraction | |
| Nitric oxide | Relaxation | |
| Oxidation-derived products | Contraction | |
| Low oxygen tension | Contraction | |
| Angiotensin II | Contraction | |
| Ascorbate | | Restores vasodilatation by acetylcholine |

tion, and with the use of the cyclo-oxygenase inhibitor meclofenamate [15]. In septic animals, both hypoxic pulmonary vasoconstriction and the effect of phenylephrine on systemic and pulmonary arteries was impaired. When meclofenamate was administered, hypoxic pulmonary vasoconstriction improved while vasoconstriction in response to phenylephrine was not changed. These data suggest that vasodilator prostaglandins may contribute to the attenuated pulmonary pressor response in sepsis.

## Endothelins

Endothelin-1 is a potent vasoconstrictor peptide [16]. During hypoxia, endothelin receptor antagonists block the hypoxic pressor response in pulmonary arteries [17]. When the endothelium is removed, the pulmonary vasoconstrictor response to hypoxia is abolished. In sepsis, blockade of endothelin-1 receptors abolishes the lipopolysaccharide (LPS)-induced pulmonary artery hypertension and worsens systemic hypotension [18].

## Angiotensin Converting Enzyme (ACE) Inhibitors

In endotoxic shock in rabbits, impaired vascular relaxation was improved by adding the ACE inhibitor perindopril [19]. The effect of perindopril depended on the availability of arginine, a substrate for NOS, and was abolished by inhibition of NOS. Hence, endothelial release of NO by perindopril seems to play an important role for re-establishment of vascular relaxation properties in resolving endotoxic shock in rabbits.

## Coagulation and Vascular Reactivity Dysfunction

This has been reviewed elsewhere [20]. Briefly, NO prevents platelet aggregation and clot formation [21]. Consequently, inhibitors of NOS have the potential to promote or enhance endotoxin-induced disseminated intravascular coagulation (DIC). In a porcine model of endotoxic shock, the administration of an NOS inhibitor resulted in coagulation abnormalities and histological changes consistent with increased activation of intravascular coagulation [22]. Alterations in coagulation during sepsis are associated with organ dysfunction and increased mortality [23].

## Vascular Reactivity during Anesthesia and Surgery

Little is known on vascular reactivity during anesthesia and surgery in humans. In pigs, the maximal *in vitro* contraction in response to norepinephrine after surgery and prolonged anesthesia is lower in hepatic as compared to superior mesenteric arteries [24]. In rats, anesthesia and surgery had profound effects on the mesenteric pressure profile; immediately after surgery, the large arteries dissipated 4% of the total pressure drop across the hepatosplanchnic circulation, arcade small arteries 16%, the intramural circulation 67%, arcade veins 9% and the remaining veins plus the hepatic circulation 7% [25]. In conscious animals, the corresponding values were 5%, 31%, 51%, and 6%. Hence, anesthesia and surgery can induce a low mesenteric flow state with the potential to induce organ damage, especially when resting perfusion is already critical or evolving hypovolemia is not appropriately treated.

## Vascular Reactivity in Sepsis, Trauma and Heart Failure

During shock, tissue hypoperfusion occurs, stimulating compensatory mechanisms in an attempt to maintain arterial pressure and organ perfusion. In this setting, vascular reactivity is modulated by physiological and inflammatory mediators, intracellular release of calcium and uptake by G protein activation. Later, the physiological vascular response (i.e., vasoconstriction) shifts towards a hypo-responsive state mediated by NO and activation of ATP-sensitive potassium channels [26]. During sepsis and severe inflammation, *in vivo* vascular reactivity is characterized by a decreased capacity to both vasodilate in response to acetycholine, and to contract in response to phenylephrine and norepinephrine (see below).

Sepsis and related syndromes release inflammatory mediators that induce vascular reactivity dysfunction, which can be associated with heterogeneous tissue perfusion and dysoxia [27–29]. Experimental data support that interleukin-1 (IL-1) plays a role in vasodilation in sepsis and during non-septic inflammation [30]. The effect of IL-1 on vascular contraction was tested using phenylephrine and potassium chloride (KCl), both with and without intact endothelium. IL-1 inhibited vascular smooth muscle contraction to both phenylephrine and KCl. This effect was not endothelium-dependent since de-endothelization did not alter the response. However, it has been shown that the effect of IL-1 on vascular reactivity is mediated by guanylate cyclase activation in response to modification of protein synthesis [31]. In vascular smooth muscle cell cultures, cGMP and oxidative products of NO such as

nitrite increased after 6 hours, with a maximum at 36 hours, when stimulated by IL-1 [32]. These effects were absent when either protein synthesis was blocked or L-arginine subtracted from the culture medium. In summary, these results suggest that during sepsis, inflammatory substances such as interleukins induce NO production in vascular smooth muscle and endothelial cells, which can mediate vascular tone and initiate tissue blood flow redistribution. Hence, the refractory vasodilation that characterizes hyperdynamic septic shock is mediated by enhanced release of NO.

Sepsis also induces endothelial-cell injury and pulmonary hypertension that promote modifications in vascular reactivity [33]. In a swine model of *Pseudomonas* sepsis, a decreased response of pulmonary vessels to KCl and impaired acetylcholine-induced relaxation was found [34]. In contrast, the response to sodium nitroprusside was not altered. However, contractility and endothelial functions decreased after five hours exposure to *Pseudomonas*. These results suggest a sepsis-induced alteration in pulmonary artery endothelial cell receptor sensitivity to acetylcholine, and a sepsis-induced alteration of muscle contraction pathways.

Regional differences of vascular reactivity have been evaluated in a rat model of sepsis [35]. In this model, the *in vitro* full contraction properties of arteries, norepinephrine response and vasorelaxation properties were tested after endotoxin infusion. The effects of endotoxemia (attenuated vasoconstriction to depolarizing KCl and the decrease of maximum contractile force with norepinephrine) were more pronounced in renal and coronary arteries than in superior mesenteric and hepatic arteries. Renal arteries showed an improvement after aminoguanidine administration and in the absence of L-arginine (a substrate for NOS). In contrast, the response of the hepatic artery was not influenced by aminoguanide or L-arginine. After 20 hours of endotoxemia, the regional variability of vascular reactivity dysfunction in response to alpha agonists increased. It has been suggested that this could be due to a regionally heterogeneous decrease in calcium sensitivity [35].

During sepsis, inflammatory mediators promote free radical production and oxidative stress [36]. It has been reported that oxidative stress plays a role in vascular dysfunction during sepsis [37]. In a mice model of cecal ligation and puncture, intravenous administration of ascorbate restored arterial responsiveness and normalized NO metabolites and inducible NOS (iNOS) expression [38]. Restoration of vascular reactivity dysfunction by ascorbate can also be explained by prevention of excessive NO production [39].

However, when endothelial NOS is blunted during sepsis, endothelium-dependent relaxation is altered. This has been demonstrated in the microcirculation and in large arteries in a rat model of polymicrobial sepsis [40]. In this model, the endothelium was selectively affected because both the contractile force after norepinephrine stimulation and relaxation in response to sodium nitroprusside were preserved. Since vasodilatation improves microcirculatory blood flow [41], inhibition of NOS does not seem to be a useful clinical option. In fact, NOS inhibition by NG-methyl-L-arginine hydrochloride has been associated with increased mortality in a clinical trial [42].

## Vascular Reactivity during Hypoxia and Low Cardiac Output States

Regional ischemia and acidosis during cardiopulmonary bypass (CPB) and in low flow states such as cardiac tamponade or cardiogenic shock may induce blood flow redistribution and/or alterations in regional oxygen extraction [43–45]. Oxygen supply and consumption are tightly regulated by vascular tone [46]. Endothelial cells react to hypoxia through a complex mechanism of depolarization and hyperpolarization that is transmitted along the vascular bed through vascular gap junctions. It has been demonstrated that hypoxia induced relaxation is mediated by NO, and that NO production is regulated by tissue $PO_2$ [47, 48].

During cardiac surgery and in low flow states, especially when vasopressor agents are used, vascular reactivity dysfunction is likely to contribute to the increased risk of mesenteric ischemia [49]. In a rat model of CPB, vascular reactivity dysfunction was associated with increased plasma levels of tumor necrosis factor (TNF)-$\alpha$ [50], suggesting an association between inflammation and vascular dysfunction even when infection is absent. In another model of hemorrhagic trauma, shock and resuscitation, mean arterial pressure was lowered to 40 mmHg, and endothelial functions were evaluated after hemorrhage had been completed and 1.5 and 4 hours following resuscitation [51]. The responses to norepinephrine and acetylcholine were both decreased at maximal bleeding, and 1.5 and 4 hours after resuscitation. In contrast, the response to nitroglycerine did not change. These results could explain the persistent and refractory hypotension despite adequate volume resuscitation after traumatic injury [51].

## Vascular Reactivity and Organ Dysfunction

Hepatic dysfunction occurs early in the course of sepsis and multiorgan dysfunction and is associated with increased morbidity and mortality [52]. Release of enzymes, accumulation of metabolic products, and coagulation disorders are late biomarkers of liver dysfunction. Early induction of hepatic dysfunction is associated with vascular reactivity dysfunction with an abnormal response to vasoactive mediators such as endothelin-1 [53]. In a model of cecal ligation and puncture, endothelin-1 infusion was associated with a distinct decrease in sinusoidal diameter and volumetric flow [53]. In addition, portal pressure increased, and high plasma alanine transferase release demonstrated hepatocellular injury. These findings suggest that sepsis enhances the effects of endothelin on vascular smooth muscles in the liver.

The effect of bacteremia with and without prior hemorrhage and resuscitation was tested in a rat model using intravital microscopy [54]. Acute bacteremia, with or without prior hemorrhage, caused significant vasoconstriction in large-caliber arterioles with concomitantly decreased blood flow. This constriction was blunted at 24 hours after hemorrhage but was completely restored by 72 hours. In pre-mucosal vessels, a marked dilation was observed both at 24 and 72 hours. Hemorrhage and bacteremia resulted in a progressive enhanced reactivity to the endothelial-dependent stimulus of acetycholine in the pre-mucosal vessels at 24 and 72 hours. Reactivity to endothelial-independent smooth muscle relaxation and subsequent vessel dilation was similar for animals with and without hemorrhage prior to bacteremia. These findings indicate that there is altered endothelial control of the intestinal mi-

crovasculature after hemorrhage in favor of enhanced dilator mechanisms in premucosal vessels with enhanced constrictor forces in inflow vessels. The data also indicate that microvascular blood flow responses to systemic inflammation can be modified by prior pathophysiological events.

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