Jejunal luminal nitric oxide during severe hypovolemia and sepsis in anesthetized pigs

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Abstract Objectives: Lowered gut blood perfusion and the associated intestinal mucosal barrier dysfunction is considered important in the pathophysiology leading to critical illness. Intestinal mucosal nitric oxide formation has been attributed a key role in the regulation of epithelial permeability and other properties of the intestinal mucosal barrier. This study was performed to delineate intestinal mucosal NO formation during hypovolemia or sepsis, both of which are associated with intestinal hypoperfusion.

Materials and methods: Seventeen pigs were subjected to 2 h of severe hypovolemia (bleeding induced) or sepsis (systemic infusion of live *Escherichia coli*) or no treatment (controls). Jejunal mucosal NO production was monitored by a tonometer. Mesenteric blood flow was measured as portal venous blood flow by an ultrasonic transit time flowmeter probe, and oxygen delivery and consumption were calculated from regional blood samples.

Results: Intestinal perfusion and oxygen delivery were reduced by the same order of magnitude in both groups. Jejunal mucosal NO production and oxygen consumption decreased markedly in the hypovolemia group but remained stable in the group subjected to septic shock.

Conclusions: These data suggest that blood loss inhibits jejunal mucosal NO production as part of a general downregulation of nonvital organs. Sepsis represents a more complex stress condition with activation/maintenance of host defense mechanisms as reflected by maintained jejunal mucosal NO production despite reduced gut blood perfusion.

Keywords Nitric oxide · Intestinal epithelium · Oxygen consumption · Splanchnic circulation · Multiple organ failure · septic shock

Introduction

Multiple organ dysfunction syndrome (MODS) is associated with high mortality [1, 2], and its obscure pathophysiology has not as yet offered cause-related therapeutic alternatives. Data from several laboratories suggest that gastrointestinal hypoperfusion is a critical event leading to MODS [3, 4, 5]. The present study was undertaken to elucidate the relationship between splanchnic hypoperfusion and jejunal mucosal nitric oxide production. The radical NO is a regulator of a multitude of gastrointestinal functions, including the mucosal barrier properties [6, 7]. For example, vascular administration of NO donors may preserve intestinal epithelial permeability [8] and microcirculation [9] during occlusive intestinal ischemia and hypovolemia. On the other hand, excessive formation of NO, as seen during inflammation and septic shock, may induce intestinal damage...
either directly by formation of cytotoxic peroxynitrite radicals [10] or indirectly by hemodynamic decompensation due to loss of systemic vascular resistance [11]. Interference with NO synthesis by the use of NOS inhibitors has been proposed as a treatment for MODS, but both beneficial and deleterious effects have been reported, indicating the complex involvement of NO in these conditions [8, 11, 12, 13, 14]. Knowledge is lacking concerning the regional, magnitude, and time relationships of NO formation. The present study assessed jejunal mucosal NO production in the jejunum of anesthetized pigs 2 h after the onset of two conditions, blood loss and sepsis, both of which are associated with gut hypoperfusion and regarded as causal factors in the development of MODS.

**Materials and methods**

Seventeen breed pigs of both sexes (mean 28 kg, range 25–32) were used in the study. The study was approved by the Committee for Ethical Review of Animal Experiments at Gothenburg University in accordance with the Swedish Animal Protection Law and conformed with the United States “Principles of Laboratory Animal Care.” All animals were fasted overnight with free access to water.

**Anesthesia**

Following induction with ketamine (Ketalar, Parke Davis, Solna, Sweden) intramuscularly (bolus, 30 mg/kg body weight) anesthesia was maintained by an intravenous infusion of α-chloralose (with a balanced pH of 7.4, Merck, Darmstadt, Germany), bolus 100 mg/kg body weight followed by 25 mg/kg per hour. Each animal was tracheostomized and mechanically ventilated (Servo 900, Siemens, Stockholm, Sweden) with air to maintain normocapnia and arterial pH around 7.4. Two heating blankets kept core body temperature at 38° to 39°C. Isotonic Ringer’s acetate solution with 2.5% glucose was infused in all animals perioperatively at the rate of 20 ml/kg per hour and during the stabilization period at a rate of 10 ml/kg per hour to maintain normovolemia as gauged by central venous pressure and pulmonary capillary wedge pressure (data not shown). Following completion of the stabilization period the fluid administration was terminated in all animals.

**Surgical preparation**

Catheters (PE 240, Kebo Laboratories, Sweden) were positioned in the left internal jugular vein and the left femoral artery and vein to monitor blood pressures and to provide vascular access for blood sampling and infusions. The right femoral artery was cannulated (PE 240, Kebo) to allow withdrawal of blood. A 7-F Swan-Ganz catheter (93A-1311H-7F, Baxter Medical, Solna, Sweden) was positioned in the pulmonary artery via the right internal jugular vein to measure cardiac output by thermodilution, which was performed via a separate injection catheter (PE 90, Kebo) positioned in a central vein. A midline laparotomy was performed, and an ultrasonic transit time flowmeter probe (inner diameter 16 mm, Transonic Systems, Ithaca, N.Y., USA) was placed around the portal vein to measure mesenteric blood flow. A catheter (PE 120, Kebo) positioned in the portal vein via a pancreatic contribu-

tory vessel was used for blood sampling. A tonometer (Tonometries, Worcester, Mass., USA) was positioned in the jejunal lumen, 50 cm aboral to the ligament of Treitz via a short antimesenteric jejunotomy and secured with a purse-string suture.

**Oxygen delivery and consumption**

Femoral arterial and portal venous blood (1 ml) was sampled in heparinized syringes and immediately analyzed (ABL 510, Radiometer, Copenhagen, Denmark) to determine hematocrit (Hct) and oxygen saturation (SO2). Mesenteric oxygen delivery (DO2) and oxygen consumption (VO2) were calculated according to:

\[
DO_2 = Hct \cdot 0.435 \cdot S_AO_2 \cdot Q_{MES} \\
VO_2 = DO_2 - (Hct \cdot 0.435 \cdot S_{pO_2} \cdot Q_{MES})
\]

where the factor 0.435 represents the product of the combining capacity of hemoglobin and the mean corpuscular hemoglobin concentration [15]. SaO2 is the arterial oxygen saturation and SpO2 is the portal venous oxygen saturation.

**NO measurements**

The gas permeable silastic balloon of the tonometer was inflated with 5 ml room air. Equilibration between NO in the intrajugular atmosphere and the air of the inflated balloon was allowed during 10 min [16]. The equilibrated gas in the balloon was then transferred into a gas-tight syringe and immediately injected into the sample line of a CLD700/AL chemiluminescence analyzer (Eco Physics, Dürnten, Switzerland) to determine the amount of NO in the sample by computing the area under the curve using the trapezoidal method for numerical integration (Power Macintosh 6100/66, Apple Computer, Cupertino, Calif., USA; LabView, National Instruments, Austin, Texas, USA). This value was used to calculate the NO concentration (parts per billion, ppb) of the tonometer [17].

**Experimental protocol**

After instrumentation all animals were left for 1–1.5 h to stabilize cardiovascular and gastrointestinal functions.

**Control animals**

This group of animals (n = 5) served as time controls and received no treatment during a 2 h control period. Intraluminal NO concentrations, hemodynamics, and oxygen delivery and consumption were measured at baseline and following 1 and 2 h (Fig. 1).

**Hypovolemia**

Hemorrhaged animals (n = 6) were subjected to graded hypovolemia by arterial withdrawal of totally 40% of the estimated total blood volume [18]. Measurements were made at baseline, after which 20% of the estimated total blood volume was withdrawn during 10 min. Measurements were repeated after a recovery period of 50 min. The animals were then bled another 20% of the estimated total blood volume over 10 min (thus reaching totally an estimated 40% loss of the blood volume at baseline). A third set of measurements were performed after additional 50 min (Fig. 1).