

Tissue concentrations of vasoactive intestinal peptide are affected by peritonitis-induced sepsis and hemofiltration in pigs

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Short title: Tissue VIP is affected by sepsis and hemofiltration

Summary

Vasoactive intestinal peptide (VIP) is a neuropeptide released from the autonomic nerves exerting multiple antiinflammatory effects. The aim of the present study was to investigate the impact of severe sepsis and hemofiltration in two settings on plasma and tissue concentrations of VIP in a porcine model of sepsis.

Thirty-two pigs were divided into 5 groups: 1. control group; 2. control group with conventional hemofiltration; 3. septic group; 4. septic group with conventional hemofiltration; 5. septic group with high-volume hemofiltration. Sepsis induced by faecal peritonitis continued for 22 hours. Hemofiltration was applied for the last 10 hours. Hemodynamic, inflammatory and oxidative stress parameters (heart rate, mean arterial pressure, cardiac output, systemic vascular resistance, plasma concentrations of tumour necrosis factor- α , interleukin-6, thiobarbituric acid reactive species, nitrate + nitrite, asymmetric dimethylarginine) and the systemic VIP concentrations were measured before faeces inoculation and at 12 and 22 hours of peritonitis. VIP tissue levels were determined in the left ventricle, mesenteric and coronary arteries.

Sepsis induced significant increases in VIP concentrations in the plasma and mesenteric artery, but it decreased peptide levels in the coronary artery. Hemofiltration in both settings reduced concentrations of VIP in the mesenteric artery.

In severe sepsis, VIP seems to be rapidly depleted from the coronary artery and, on the other hand, upregulated in the mesenteric artery. Hemofiltration in both settings has tendency to drain away these upregulated tissue stores which could result in the limited secretory capacity of the peptide.

Key words:

VIP plasma levels, severe sepsis, coronary artery, mesenteric artery, heart

Introduction

Vasoactive intestinal peptide (VIP) is an octacosapeptide released from the autonomic nerves acting as a nonadrenergic noncholinergic neurotransmitter or neuromodulator. Significant concentrations of VIP are present in the gastrointestinal, cardiovascular, respiratory and urogenital systems (Lundberg 1996). Many systemic and pulmonary blood vessels are innervated by nerve fibres releasing VIP that causes profound and long-lasting relaxation of the vascular smooth muscle (Champion *et al.* 1996). In the mammalian heart, VIP immunoreactivity has been demonstrated in cholinergic neurones that release the peptide upon high frequency electrical stimulation of the vagus nerve and mediate positive chronotropic effect known as vagal tachycardia (Henning and Sawmiller 2001). In addition, VIP seems to have protective action during acute myocardial ischemia acting as a free-radical scavenger, decreasing myocardial release of creatinine kinase and improving cardiac perfusion (Kalfin *et al.* 1994).

Besides its role in the regulation of the major cardiovascular variables, VIP emerges as an important contributor to neuroimmune crosstalk: nerve fibres containing VIP supply lymphoid organs (Bellinger *et al.* 1990; Kulkarni-Narla *et al.* 1999), white blood cells express VIP receptors (Ganea and Delgado 2002) and might produce also the peptide itself especially under inflammatory stimuli (Kawashima *et al.* 2007). VIP attenuates deleterious consequences of sepsis-induced macrophage activation by inhibiting tumour necrosis- α and interleukin-6 production and stimulating release of anti-inflammatory cytokine IL-10 (Delgado *et al.* 1999). Furthermore, VIP is involved in neutrophil recruitment in different target organs (Martínez *et al.* 2006), inhibits tissue factor expression in monocytes (Lv *et al.* 2009) and seems to be protective even in the later phases of sepsis by limiting secretion of the high mobility group box 1 protein (Chorny and Delgado 2008). In vivo data documented VIP-induced protection from lethal peritonitis in mice and increased susceptibility to death from endotoxemia in VIP gene knockout mice (Hamidi *et al.* 2006). In septic patients, increased VIP systemic levels have been demonstrated (Brandtzaeg *et al.* 1989). Several recently

published papers suggest that VIP could be an attractive candidate for the development of therapies against severe sepsis and septic shock (Martínez *et al.* 2005; Toscano *et al.* 2010). Severe sepsis is characterized by dysregulated host response to infection associated with an excessive production and release of both pro- and anti-inflammatory cytokines and consecutive multiple organ dysfunction (Hotchkiss and Karl 2003). Therapeutic strategies aiming at reducing cytokine levels elevated by the systemic inflammation in sepsis tested so far challenged either single specific inflammatory agent using monoclonal antibodies or acted nonspecifically, by eliminating excess of mediators released into the blood compartment in relation to the septic status (Ronco *et al.* 2003). Whereas single-substance strategies did not show anticipated beneficial effects and had even harmful consequences (Dinarello 2001), hemofiltration and related techniques seem to be more promising, particularly with regard to the hemodynamic stability and need for vasopressors support (Ratanarat *et al.* 2005). In spite of these potential clinical benefits, so far reported impact of hemopurification techniques on the plasma levels of various noxious substances and downstream biological pathways implicated in sepsis pathophysiology is still equivocal (Reeves *et al.* 1999; Sykora *et al.* 2009). In addition, considerable attention has been focused on benefits of hemofiltration, whereas less attention has been paid to the potential for increased risk. Indeed, non-selective detoxification not only might remove harmful substances but also water soluble molecules with potentially beneficial effects.

The aim of the present study was to verify sepsis-induced elevations in plasma VIP levels in a clinically relevant porcine model of hyperdynamic septic shock induced by fecal peritonitis and to investigate putative impact of hemofiltration in two settings on the plasma and tissue concentrations of VIP.

Methods

Thirty-two domestic pigs (Farm Mladotice, Czech Republic) of both sexes weighing 30 – 45 kg were studied. All experiments were conducted in accordance with the relevant Guidelines of the Czech Ministry of Agriculture for Scientific Experimentation on Animals and the

European Directive for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (86/609/EEC) and were approved by the University Committee for Experiments on Laboratory Animals (Charles University, Czech Republic). Pigs were divided into 5 groups: 1. control group without hemofiltration (n = 6); 2. control group subjected to conventional hemofiltration (ultrafiltration rate 35 ml/kg/hr, n = 4); 3. septic group without hemofiltration (n = 8); 4. septic group with conventional hemofiltration (ultrafiltration rate 35 ml/kg/hr, n = 6); and 5. septic group undergoing high-volume hemofiltration (ultrafiltration rate 100 ml/kg/hr, n = 8). The anesthesia, surgery and physiological measurements were performed as described recently (Chvojka *et al.* 2008). Briefly, anesthesia was induced by propofol (1-2 mg/kg) and ketamine (2 mg/kg) and maintained by thiopental (5 mg/kg/h) and fentanyl (5 µg/kg/h). Animals were mechanically ventilated with arterial pCO₂ maintained between 4 and 5 kPa. Central venous catheter, pulmonary artery thermodilution catheter, femoral artery and femoral vein catheters were inserted for drug and fluid infusion, cardiac output and blood pressure recordings and hemofiltration access, respectively. Noradrenaline was administered to maintain mean arterial pressure greater than or equal to 65 mmHg. Fluid resuscitation was guided by continuous cardiac filling pressures and intrathoracic blood volume measurements.

The hemofiltration was performed on the MultiFiltrate system (Fresenius Medical Care AG & Co. KGaA, Bad Homburg, Germany) with a synthetic membrane (Ultraflux AV600S; the high-flux polysulphone filter, surface 1.4 m², cut off up to 30,000 Dalton, Fresenius). Continuous veno-venous hemofiltration and high-volume continuous veno-venous hemofiltration with zero-balanced treatment were set at ultrafiltration rates of 35 and 100 ml/kg/hour, respectively. Blood flow rate was set at 200 ml/min. Bicarbonate buffered (35 mmol/l) replacement fluid (Medisol Bi-4, solution for hemofiltration, Medites Pharma, Rožnov pod Radhoštěm, Czech Republic) was administered 50% in predilution and 50% in postdilution fashion. The replacement fluid was warmed to 39°C in the MultiFiltrate, and the HOTLINE[®] Blood and Fluid Warmer (Smiths Medical, Herts, United Kingdom) was inserted on the venous return line to prevent an extracorporeal circuit-induced decrease in body

temperature. Anticoagulation was achieved with an initial i.v. bolus 0.2 ml/10 kg of nadroparinum (Fraxiparine, Glaxo Group Ltd., Greenford, Middlesex, United Kingdom) for all experimental animals, including the control groups.

Baseline data were recorded after six hours of postsurgical stabilization period; peritonitis was induced by autologous faeces inoculation and second set of measurements was obtained 12 hours later. In hemofiltration groups, continuous conventional or high-volume hemofiltration was started at 12 hours and continued for additional 10 hours till the end of experiment. The final set of data, blood and ultrafiltrate samples were taken in the end of hemofiltration procedure i.e. at 22 hours of peritonitis. The animals were euthanized by KCl injection under deep anaesthesia.

Hemodynamic measurements included heart rate (electrocardiography), systolic and diastolic blood pressures (electronic pressure transducer), and cardiac output (thermodilution technique). Systemic vascular resistance was calculated as (mean arterial pressure – central venous pressure)/cardiac output. Concentrations of inflammatory cytokines TNF- α and IL-6 in the plasma were determined by commercial immunoassay diagnostic kits (Biosource swine TNF- α ELISA, Biosource International, Inc., Camarillo, CA, USA and Quantikinine, Porcine IL-6 immunoassay, R&D Systems, Inc., Minneapolis, MN, USA, respectively).

Oxidative and nitrosative stress was evaluated by determination of arterial thiobarbituric acid reactive species concentrations (TBARS) by spectrophotometric method (Chvojka et al 2008), arterial nitrate + nitrite (NO_x) concentrations (colorimetric method; Matejovic et al. 2004), and asymmetric dimethylarginine (ADMA; commercial enzyme-linked immunosorbent assay kits ADMA® ELISA, DLD Diagnostika GmbH, Hamburg, Germany).

VIP levels in the arterial plasma, ultrafiltrate and extracts of the proximal left coronary artery, mesenteric artery and left ventricular wall were determined by commercial radioimmunoassay kits (Phoenix Pharmaceuticals Inc., Burlingame, CA, USA) after extraction and purification with equilibrated SEP-Pak C18 cartridges (RK-SEPCOL-1; Phoenix Pharmaceuticals Inc., Burlingame, CA, USA).

Results are presented as median and 25th and 75th percentiles. TNF- α , IL-6, TBARS, NO_x, ADMA and VIP levels in the blood or plasma were normalized for plasma protein content to correct dilutional effects resulting from volume resuscitation (Matejovic *et al.* 2004). Tissue concentrations of VIP were expressed in ng/g or pg/g tissue wet weight where appropriate. Statistical differences were analyzed using software package STATISTICA Cz, version 7 (StatSoft Inc., 2004, Prague, Czech Republic). After testing for the normality of distribution and homogeneity of variances, comparisons of parametric variables were made using two-way ANOVA or repeated measures ANOVA followed by post hoc Fisher's Least Significant Difference test. Nonparametric variables were compared using the Friedman ANOVA on ranks or Kruskal-Wallis ANOVA where appropriate. The results were considered significantly different when $P < 0.05$.

Results

Cardiovascular variables

Hemodynamic parameters are listed in Table 1. There were no statistically significant differences in any of the measured variables among the five groups prior to induction of peritonitis (baseline). Inoculation of autologous faeces resulted in hyperdynamic septic circulation with a high cardiac output and low systemic vascular resistance. No significant impact of hemofiltration on the hemodynamic variables could be observed in the control pigs. Likewise, septic animals undergoing hemofiltration procedures in both settings did not display any additional differences in hemodynamic parameters compared to septic pigs without hemofiltration.

Inflammation, oxidative and nitrosative stress

Table 2 shows the systemic levels of TNF- α , IL-6, TBARS, NO_x and ADMA in the five groups. Significant differences were observed between the baseline and septic values. There were no significant changes in the monitored variables in the control animals over

the time. Hemofiltration did not affect any of the measured parameters in both control and septic animals.

VIP levels in the plasma, ultrafiltrates, and tissues

In all control animals (both subjected and not to hemofiltration), systemic VIP concentrations did not markedly change over the time (data not shown). Sepsis led to significant elevations of the peptide levels in the arterial plasma that slightly declined toward the end of the experiment remaining still significantly higher than the baseline values (Fig. 1A). Similar trends were observed in septic pigs subjected to conventional and high-volume hemofiltrations.

VIP was detected in the ultrafiltrates from both conventional and high-volume hemofiltrations. Estimated cumulative losses of VIP into ultrafiltrate were 46 ng/10 h in the control animals subjected to conventional hemofiltration, 57 ng/10 h and 145 ng/10 h in septic pigs subjected to conventional and high-volume hemofiltration, respectively.

As shown in Figure 1B, concentrations of VIP in the coronary artery were significantly decreased in all septic pigs and they were not further affected by hemofiltration. Interestingly, there was a trend for conventional hemofiltration to reduce VIP levels in the coronary artery of the control animals ($P = 0.06$). Mesenteric arteries isolated from the septic pigs not subjected to hemofiltration displayed significantly increased VIP levels compared to control pigs without hemofiltration (Fig. 1C). Hemofiltration resulted in ~34% reduction in the tissue content of the peptide in the mesenteric arteries of the control animals ($P < 0.05$ vs controls without hemofiltration); in the septic pigs, hemofiltration led to arterial tissue levels not differing from the control values. VIP concentrations in the myocardial tissue did not differ among all groups (Fig. 1D).

Discussion

Effect of sepsis on VIP plasma and tissue levels

The present study addressed the impact of peritonitis-induced septic shock on VIP concentrations in the plasma and reports, for the first time, sepsis- and hemofiltration-related levels of VIP in the selected cardiovascular tissues of the pig. The results confirmed a transient increase in the systemic VIP concentrations previously reported in endotoxemic pigs, dogs and in human patients suffering from gram-negative septic shock (Brandtzaeg *et al.* 1989; Fuortes *et al.* 1988; Revhaug *et al.* 1985). The decline in VIP plasma concentrations between 12 and 22 hours of the sepsis duration could reflect the putative changes in a delicate balance between the synthesis of the peptide and its transport to storage sites, peptide release induced by the systemic inflammation and its degradation. It has been recently reported that the activity of VIP-degrading enzyme, neutral endopeptidase, is diminished in the septic patients, which contributes to the decreased clearance of degraded peptides thus at least partly compensating for their increased need (Pirracchio *et al.* 2008).

It should be noted that neuropeptides, in contrast to classical neurotransmitters, are not synthesized in the nerve endings, but in the neuronal sommata and then they are transported to the site of their release by axonal transport. Realizing that the transport rate of VIP to nerve terminals is approximately 9 mm/h and that the half-life of the peptide in the plasma is only 1 min (Henning and Sawmiller 2001), the decreasing plasma levels of the peptide could be associated with its compromised release from at least partially depleted tissue stores. Circulating VIP is derived mainly from the enteric nervous system and vascular nerves that display variable density of the peptide-containing nerve fibers from rare (vessels in the skeletal muscles) to moderate (coronary arteries) and intense (mesenteric vasculature) (Opstad 1987; Owman 1990). VIP-ergic nerve fibers supplying the mesenteric artery in the pig extend from the enteric neurons (Shen *et al.* 1993) that display considerable plasticity in response to inflammation (Birch *et al.* 2008; Jacob *et al.* 2007; Pidsudko *et al.* 2008). Our study shows a significant increase in VIP concentration in the mesenteric artery of pig with peritonitis-induced sepsis, which could result from the progression of intraabdominal inflammation induced by inoculation of faeces. It has been already documented that bowel

perforation leads to increased VIP expression in the myenteric plexus of human patients suffering from subsequent peritonitis (Jacob *et al.* 2007).

In contrast, coronary arteries of the septic pigs displayed significantly lower peptide levels than the respective controls. Concentrations of VIP in the left ventricular myocardium were negligible in both septic and control animals. In the heart, VIP originates from the parasympathetic postganglionic fibers and intrinsic nonadrenergic noncholinergic neuronal network (Anderson *et al.* 1993). The cardiac tissue does not normally metabolize VIP thus contributing to the systemic VIP levels by its lymphatic and coronary venous drainage (Anderson *et al.* 1993). Decreased concentration of VIP in the coronary artery after 22 hours of sepsis suggests that the peptide stores could be depleted within relatively short duration of the systemic inflammation and that VIP cannot be released from the coronary circulation in substantial amounts in further course of sepsis.

Impact of blood purification techniques on VIP plasma and tissue levels

Our study also indicates that hemofiltration in both settings did not significantly affect plasma VIP concentrations in any experimental group; however, a trend to decrease VIP levels in the vascular tissue could be observed both in control and septic animals.

VIP belongs to low molecular weight peptides (MW 3300 Da; Clark *et al.* 1999) that should be susceptible to filtration through the filter used in this study (cut off 30,000 Da). Indeed, after appropriate extraction and concentration, we have detected significant amounts of VIP in the ultrafiltrates that, when compared to peptide tissue content, could lead to at least partial depletion of VIP from its storage sites. Previously published studies on cytokine removal by continuous venovenous hemofiltration under conditions of sepsis mainly reported no change in plasma cytokine levels (Heering *et al.* 1997; Heering *et al.* 2003; Sykora *et al.* 2009) in spite of detectable levels of cytokines in the ultrafiltrate (Jiang *et al.* 2005) or their adsorption on the ultrafiltration membrane (Goldfarb and Golper 1994). These studies dealt with cytokines with substantially greater molecular weights than is that of VIP and they did not address cytokine tissue levels.

Two hypotheses have been suggested to explain the discrepancy between apparent removal of inflammatory cytokines by hemopurification techniques and no change in their systemic levels: in the threshold immunomodulation hypothesis proposed by Honoré and collaborators, both pro- and antiinflammatory mediators are removed at the interstitial and tissue levels and hemofiltration might facilitate mediator flow from tissues to the blood compartment (Honoré *et al.* 2007). In the mediator delivery hypothesis (Di Carlo and Alexander 2005), high-volume hemofiltration is thought to promote the lymphatic flow thus causing drag of mediators to the blood compartment for subsequent removal. Both theories suggest that significant amounts of mediators are removed from the sites of their production or accumulation.

Study limitations

Our study has some limitations related to the model designed to induce hyperdynamic sepsis with increasing severity over time, where standard antibiotic therapy that would attenuate the inflammatory response was not used. Therefore, the observation study time was relatively short (i.e. 22 hours) not allowing us to monitor VIP plasma and tissue levels in the later stages of sepsis, which could provide better insight into the changes of the peptide stores in relation to the course of the systemic inflammatory process. The true clinical consequences of the suspected peptide depletion due to hemofiltration could not be derived from the present data. However, remarkable effects of the peptide and its receptor agonists in the protection against endotoxemia has been repeatedly shown in experiments using *in vivo* VIP administration, inoculation of cells producing VIP, VIP receptor agonists and antagonists and VIP gene knockout mice that clearly demonstrated not only inhibitory effect of VIP and the related compounds on the release of TNF- α , IL-6, IL-12 and NO, but also significant increase in the survival rate of treated endotoxemic mice (Chorny and Delgado 2008; Delgado *et al.* 1999; Hamidi *et al.* 2006; Matínez *et al.* 2005). Nevertheless, relation of VIP plasma levels to the survival rate in human septic patients has not been established yet.

Conclusion

Taken together, this is the first report documenting the impact of hemofiltration on tissue stores of a substance that has multiple beneficial effects in body's defense against infection. Under septic conditions, peptide seems to be rapidly depleted from the coronary artery and, on the other hand upregulated in the mesenteric artery. Hemofiltration in both settings has tendency to drain away these upregulated tissue stores which could result in the limited secretory capacity of the peptide.

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References

1. ANDERSON FL, KRALIOS AC, REID B, HANSON GR: Release of vasoactive intestinal peptide and neuropeptide Y from canine heart. *Am J Physiol* **265**: H959-H965, 1993.
2. BELLINGER DL, LORTON D, ROMANO TD, OLSCHOWKA JA, FELTEN SY, FELTEN DL: Neuropeptide innervation of lymphoid organs. *Ann N Y Acad Sci* **594**: 17-33, 1999.
3. BIRCH D, KNIGHT GE, BOULOS PB, BURNSTOCK G: Analysis of innervation of human mesenteric vessels in non-inflamed and inflamed bowel--a confocal and functional study. *Neurogastroenterol Motil* **20**: 660-670, 2008.
4. BRANDTZAEG P, OKTEDALEN O, KIERULF P, OPSTAD PK: Elevated VIP and endotoxin plasma levels in human gram-negative septic shock. *Regul Pept* **24**: 37-44, 1989.
5. CHAMPION HC, SANTIAGO JA, GARRISON EA, CHENG DY, COY DH, MURPHY WA, ASCUITTO RJ, ROSS-ASCUITTO NT, MCNAMARA DB, KADOWITZ PJ: Analysis of cardiovascular responses to PACAP-27, PACAP-38, and vasoactive intestinal polypeptide. *Ann N Y Acad Sci* **805**: 429-441, 1996.

6. CHORNY A, DELGADO M: Neuropeptides rescue mice from lethal sepsis by down-regulating secretion of the late-acting inflammatory mediator high mobility group box 1. *Am J Pathol* **172**: 1297-1307, 2008.
7. CHVOJKA J, SYKORA R, KROUZECKY A, RADEJ J, VARNEROVA V, KARVUNIDIS T, HES O, NOVAK I, RADERMACHER P, MATEJOVIC M: Renal haemodynamic, microcirculatory, metabolic and histopathological responses to peritonitis-induced septic shock in pigs. *Crit Care* **12**: R164, 2008.
8. CLARK WR, HAMBURGER RJ, LYSAGHT MJ: Effect of membrane composition and structure on solute removal and biocompatibility in hemodialysis. *Kidney Int* **56**: 2005-2015, 1999.
9. DELGADO M, POZO D, MARTINEZ C, LECETA J, CALVO JR, GANEA D, GOMARIZ RP: Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide inhibit endotoxin-induced TNF-alpha production by macrophages: in vitro and in vivo studies. *J Immunol* **162**: 2358-2367, 1999.
10. DI CARLO JV, ALEXANDER SR: Hemofiltration for cytokine-driven illnesses: the mediator delivery hypothesis. *Int J Artif Organs* **28**: 777-786, 2005.
11. DINARELLO CA: Anti-cytokine therapies in response to systemic infection. *J Invest Dermatol Symp Proc* **6**: 244-250, 2001.
12. FUORTES M, BLANK MA, SCALEA TM, POLLOCK TW, JAFFE BM: Release of vasoactive intestinal peptide during hyperdynamic sepsis in dogs. *Surgery* **104**: 894-898, 1988.
13. GANEA D, DELGADO M: Vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP) as modulators of both innate and adaptive immunity. *Crit Rev Oral Biol Med* **13**: 229-237, 2002.
14. GOLDFARB S, GOLPER TA: Proinflammatory cytokines and hemofiltration membranes. *J Am Soc Nephrol* **5**: 228-232, 1994.

15. HAMIDI SA, SZEMA AM, LYUBSKY S, DICKMAN KG, DEGENE A, MATHEW SM, WASCHEK JA, SAID SI: Clues to VIP function from knockout mice. *Ann N Y Acad Sci* **1070**: 5-9, 2006.
16. HEERING P, GRABENSEE B, BRAUSE M: Cytokine removal in septic patients with continuous venovenous hemofiltration. *Kidney Blood Press Res* **26**: 128-134, 2003.
17. HEERING P, MORGERA S, SCHMITZ FJ, SCHMITZ G, WILLERS R, SCHULTHEISS HP, STRAUER BE, GRABENSEE B: Cytokine removal and cardiovascular hemodynamics in septic patients with continuous venovenous hemofiltration. *Intensive Care Med* **23**: 288-296, 1997.
18. HENNING RJ, SAWMILLER DR: Vasoactive intestinal peptide: cardiovascular effects. *Cardiovasc Res* **49**: 27-37, 2001.
19. HONORÉ PM, JOANNES-BOYAU O, GRESSENS B: Blood and plasma treatments: the rationale of high-volume hemofiltration. *Contrib Nephrol* **156**: 387-395, 2007.
20. HOTCHKISS RS, KARL IE: The pathophysiology and treatment of sepsis. *N Engl J Med* **348**: 138-150, 2003.
21. JACOB P, MUELLER MH, HAHN J, WOLK I, MAYER P, NAGELE U, HENNENLOTTER J, STENZL A, KONIGSRAINER A, GLATZLE J: Alterations of neuropeptides in the human gut during peritonitis. *Langenbecks Arch Surg* **392**: 267-271, 2007.
22. JIANG HL, XUE WJ, LI DQ, YIN AP, XIN X, LI CM, GAO JL: Influence of continuous veno-venous hemofiltration on the course of acute pancreatitis. *World J Gastroenterol* **11**:4815-4821, 2005.
23. KALFIN R, MAULIK N, ENGELMAN RM, CORDIS GA, MILENOV K, KASAKOV L, DAS DK: Protective role of intracoronary vasoactive intestinal peptide in ischemic and reperfused myocardium. *J Pharmacol Exp Ther* **268**: 952-958, 1994.
24. KAWASHIMA K, YOSHIKAWA K, FUJII YX, MORIWAKI Y, MISAWA H: Expression and function of genes encoding cholinergic components in murine immune cells. *Life Sci* **80**: 2314-2319, 2007.

25. KULKARNI-NARLA A, BEITZ AJ, BROWN DR: Catecholaminergic, cholinergic and peptidergic innervation of gut-associated lymphoid tissue in porcine jejunum and ileum. *Cell Tissue Res* **298**: 275-286, 1999.
26. LUNDBERG JM: Pharmacology of cotransmission in the autonomic nervous system: integrative aspects on amines, neuropeptides, adenosine triphosphate, amino acids and nitric oxide. *Pharmacol Rev* **48**: 113-178, 1996.
27. LV B, TANG Y, CHEN F, XIAO X: Vasoactive Intestinal Peptide and pituitary adenylate cyclase-activating polypeptide inhibit tissue factor expression in monocyte in vitro and in vivo. *Shock* **31**: 185-191, 2009.
28. MARTÍNEZ C, ARRANZ A, JUARRANZ Y, ABAD C, GARCÍA-GÓMEZ M, ROSIGNOLI F, LECETA J, GOMARIZ RP: PAC1 receptor: emerging target for septic shock therapy. *Ann N Y Acad Sci* **1070**: 405-410, 2006.
29. MARTÍNEZ C, JUARRANZ Y, ABAD C, ARRANZ A, MIGUEL BG, ROSIGNOLI F, LECETA J, GOMARIZ RP: Analysis of the role of the PAC1 receptor in neutrophil recruitment, acute-phase response, and nitric oxide production in septic shock. *J Leukoc Biol* **77**: 729-738, 2005.
30. MATEJOVIC M, KROUZECKY A, MARTINKOVA V, ROKYTA R JR, KRALOVA H, TRESKA V, RADERMACHER P, NOVAK I: Selective inducible nitric oxide synthase inhibition during long-term hyperdynamic porcine bacteremia. *Shock* **21**: 458-465, 2004.
31. OPSTAD PK: The plasma vasoactive intestinal peptide (VIP) response to exercise is increased after prolonged strain, sleep and energy deficiency and extinguished by glucose infusion. *Peptides* **8**: 175-178, 1987.
32. OWMAN C: Peptidergic vasodilator nerves in the peripheral circulation and in the vascular beds of the heart and brain. *Blood Vessels* **27**: 73-93, 1990.
33. PIDSUDKO Z, KALECZYC J, WASOWICZ K, SIENKIEWICZ W, MAJEWSKI M, ZAJAC W, LAKOMY M: Distribution and chemical coding of intramural neurons in the porcine ileum during proliferative enteropathy. *J Comp Pathol* **138**: 23-31, 2008.

34. PIRRACCHIO R, DEYE N, LUKASZEWICZ AC, MEBAZAA A, CHOLLEY B, MATÉO J, MÉGARBANE B, LAUNAY JM, PEYNET J, BAUD F, PAYEN D: Impaired plasma B-type natriuretic peptide clearance in human septic shock. *Crit Care Med* **36**: 2542-2546, 2008.
35. RATANARAT R, BRENDOLAN A, PICCINNI P, DAN M, SALVATORI G, RICCI Z, RONCO C: Pulse high-volume haemofiltration for treatment of severe sepsis: effects on hemodynamics and survival. *Crit Care* **9**: R294-R302, 2005.
36. REEVES JH, BUTT WW, SHANN F, LAYTON JE, STEWART A, WARING PM, PRESNEILL JJ: Continuous plasmafiltration in sepsis syndrome. Plasmafiltration in Sepsis Study Group. *Crit Care Med* **27**: 2096-2104, 1999.
37. REVHAUG A, LYGREN I, LUNDGREN TI, ANDERSEN OK, BURHOL PG, GIERCKSKY KE: Increased plasma levels of vasoactive intestinal polypeptide in pigs during endotoxinaemia. *Eur Surg Res* **17**: 75-82, 1985.
38. RONCO C, TETTA C, MARIANO F, WRATTEN ML, BONELLO M, BORDONI V, CARDONA X, INGUAGGIATO P, PILOTTO L, D'INTINI V, BELLOMO R: Interpreting the mechanisms of continuous renal replacement therapy in sepsis: the peak concentration hypothesis. *Artif Organs* **27**: 792-801, 2003.
39. SHEN Z, KLÖVER-STÅHL B, LARSSON LT, MALMFORS G, EKBLAD E, SUNDLER F: Peptide-containing neurons remain unaffected after intestinal autotransplantation: an experimental study in the piglet. *Eur J Pediatr Surg* **3**: 271-277, 1993.
40. SYKORA R, CHVOJKA J, KROUZECKY A, RADEJ J, KARVUNIDIS T, VARNEROVA V, NOVAK I, MATEJOVIC M: High versus standard-volume haemofiltration in hyperdynamic porcine peritonitis: effects beyond haemodynamics? *Intensive Care Med* **35**: 371-380, 2009.
41. TOSCANO MG, DELGADO M, KONG W, MARTIN F, SKARICA M, GANEA D: Dendritic Cells Transduced With Lentiviral Vectors Expressing VIP Differentiate Into VIP-secreting Tolerogenic-like DCs. *Mol Ther* **18**: 1035-1045, 2010.

TABLES AND FIGURES

TABLE 1. Systemic hemodynamics

		Baseline	12 hours	22 hours
MAP [mmHg]	CON-NO HF	91 (86; 97)	82 (74; 83)	80 (74; 84)
	CON-CHF	95 (89; 104)	97 (92; 102)	89 (77; 101)
	SEP-NO HF	94 (89; 103)	95 (88; 106)	80 (75; 97)
	SEP-CHF	83 (82; 92)	90 (84; 94)	76 (71; 80)
	SEP-HVHF	98 (92; 106)	88 (78; 110)	71 (66; 81)*
CO [ml.kg ⁻¹ .min ⁻¹]	CON-NO HF	85 (80; 104)	93 (77; 99)	79 (67; 96)
	CON-CHF	91 (79; 97)	81 (71; 99)	76 (68; 85)
	SEP-NO HF	78 (61; 90)	105 (97; 119)*	129 (104; 180)*
	SEP-CHF	77 (72; 81)	130 (114; 136)*	155 (133; 164)*
	SEP-HVHF	85 (71; 104)	110 (102; 119)	162 (149; 168)*
HR [min ⁻¹]	CON-NO HF	82 (67; 85)	92 (83; 94)	89 (82; 93)
	CON-CHF	85 (75; 95)	106 (103; 111)	113 (106; 122)
	SEP-NO HF	53 (52; 67)	121 (111; 136)*	166 (158; 180)*
	SEP-CHF	75 (65; 83)	126 (112; 128)*	144 (133; 155)*
	SEP-HVHF	66 (63; 81)	112 (108; 123)*	167 (119; 173)*
SVR [dyne.sec.cm ⁻⁵]	CON-NO HF	2535 (1761; 2823)	2007 (1924; 2084)	2136 (1922; 2283)
	CON-CHF	2355 (2160; 2588)	2551 (2061; 2893)	2296 (1795; 2879)
	SEP-NO HF	2756 (2318; 2830)	1779 (1579; 1978)*	1001 (795; 1643)*
	SEP-CHF	2127 (1914; 2584)	1260 (1191; 1309)*	892 (858; 1113)*
	SEP-HVHF	2507 (2144; 2739)	1891 (1598; 1982)*	750 (665; 974)*

MAP, mean arterial pressure; CO, cardiac output; HR, heart rate; SVR, systemic vascular

resistance in the control pigs without hemofiltration (CON-NO HF), control pigs with

conventional hemofiltration (CON-CHF), septic pigs without hemofiltration (SEP-NO HF),

septic pigs with conventional hemofiltration (SEP-CHF), septic pigs with high-volume

hemofiltration (SEP-HVHF). *Significant difference within each group vs baseline ($p < 0.05$).

Data are median and 25th and 75th percentiles.

TABLE 2. Inflammation and oxidative/nitrosative stress

		Baseline	12 hours	22 hours
IL-6 [ng/g protein]	CON-NO HF	1.8 (1.2; 2.3)	0.7 (0.3; 1.2)	1.2 (0.5; 1.6)
	CON-CHF	4.4 (2.9; 7.1)	1.5 (1.1; 2.2)	1.3 (1.0; 1.4)
	SEP-NO HF	2.7 (1.6; 3.8)	26.7 (10.6; 29.2)*	86.3 (26.6; 481.7)*
	SEP-CHF	2.2 (0.6; 3.9)	13.7 (11.2; 21.7)*	128.0 (47.4; 210.1)*
	SEP-HVHF	2.8 (2.1; 6.4)	36.4 (19.0; 71.7)*	857.8 (65.5; 1559.3)*
TNF- α [ng/g protein]	CON-NO HF	0.8 (0.7; 0.9)	1.3 (1.2; 1.5)	1.4 (1.2; 1.4)
	CON-CHF	1.5 (1.3; 1.6)	2.0 (1.5; 2.5)	0.9 (0.8; 1.1)
	SEP-NO HF	1.4 (1.1; 1.4)	4.7 (4.1; 6.4)*	12.8 (4.9; 21.8)*
	SEP-CHF	1.0 (0.4; 1.7)	4.8 (2.8; 7.2)*	13.5 (8.9; 13.5)*
	SEP-HVHF	1.6 (0.8; 5.6)	6.8 (4.6; 9.8)*	7.0 (5.3; 12.3)*
TBARS [pmol/g protein]	CON-NO HF	16.8 (16.4; 18.4)	20.4 (17.4; 27.8)	22.8 (16.3; 23.1)
	CON-CHF	19.2 (17.8; 21.0)	23.2 (20.5; 24.6)	23.2 (21.7; 27.0)
	SEP-NO HF	18.5 (15.4; 21.1)	46.6 (39.1; 57.7)*	75.2 (56.8; 98.1)*
	SEP-CHF	17.8 (14.9; 19.3)	43.1 (34.7; 47.0)*	52.9 (44.7; 78.2)*
	SEP-HVHF	17.1 (15.2; 23.4)	47.0 (35.4; 53.8)*	83.6 (52.8; 109.2)*
Nitrite+nitrate [μ mol/g protein]	CON-NO HF	0.9 (0.7; 1.2)	0.6 (0.5; 1.0)	0.7 (0.5; 1.1)
	CON-CHF	1.0 (0.6; 1.7)	0.9 (0.7; 1.1)	0.8 (0.6; 1.0)
	SEP-NO HF	0.7 (0.6; 0.9)	1.2 (1.0; 1.5)*	1.3 (1.0; 1.5)*
	SEP-CHF	0.6 (0.5; 0.8)	1.3 (0.9; 1.7)*	1.1 (1.0; 1.2)
	SEP-HVHF	0.9 (0.4; 1.5)	1.7 (1.3; 1.9)*	1.6 (1.4; 1.6)
ADMA [nmol/g protein]	CON-NO HF	51.5 (49.5; 53.5)	47.0 (45.0; 59.5)	54.0 (48.5; 62.5)
	CON-CHF	72.5 (65.5; 79.8)	53.5 (44.5; 61.8)	52.0 (32.2; 83.3)
	SEP-NO HF	58.5 (47.2; 70.8)	85.0 (75.2; 92.8)*	68.0 (48.5; 100.0)
	SEP-CHF	49.0 (42.5; 57.8)	55.0 (44.0; 79.5)	76.0 (40.8; 111.3)*
	SEP-HVHF	62.0 (48.3; 67.8)	75.0 (66.8; 115.3)*	94.0 (65.3; 114.3)*

IL-6, interleukin-6; TNF- α , tumour necrosis factor- α , TBARS, thiobarbituric acid reactive species; ADMA, asymmetric dimethylarginine concentrations in the plasma of the control pigs without hemofiltration (CON-NO HF), control pigs with conventional hemofiltration

(CON-CHF), septic pigs without hemofiltration (SEP-NO HF), septic pigs with conventional hemofiltration (SEP-CHF), septic pigs with high-volume hemofiltration (SEP-HVHF).

*Significant difference within each group vs baseline ($p < 0.05$). Data are median and 25th and 75th percentiles.

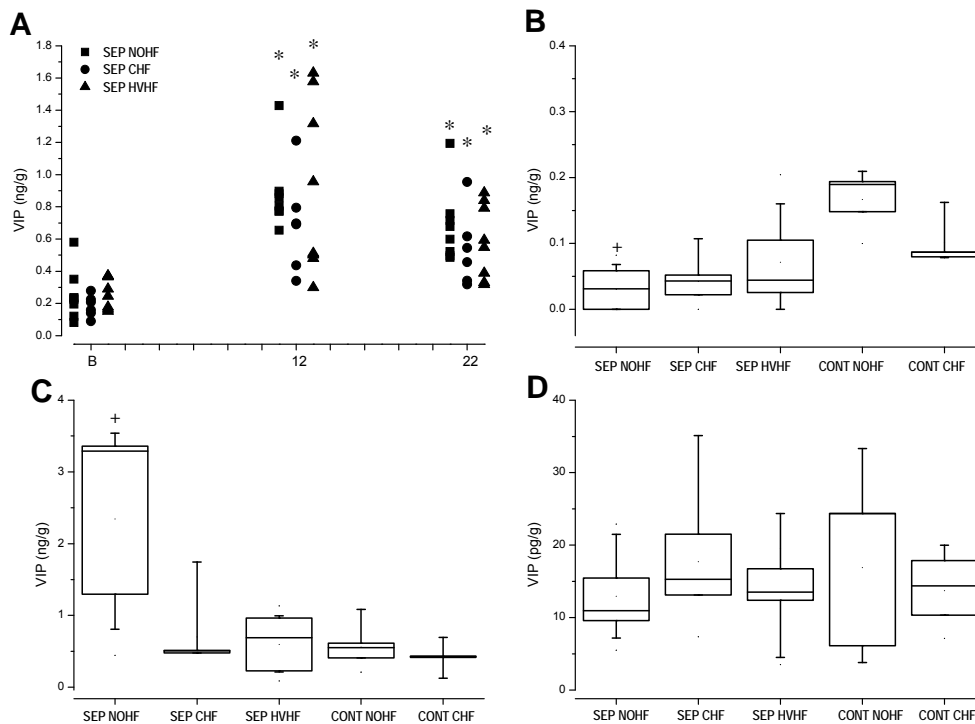


FIGURE 1A. VIP concentrations in the plasma of the septic pigs without hemofiltration (SEP-NO HF), septic pigs with conventional hemofiltration (SEP-CHF), septic pigs with high-volume hemofiltration (SEP-HVHF). *Significant difference vs. baseline (B; $p < 0.05$).

B-D. VIP concentrations in the coronary artery (B), mesenteric artery (C), and left ventricular free wall (D), in the control pigs without hemofiltration (CON-NO HF), control pigs with conventional hemofiltration (CON-CHF), septic pigs without hemofiltration (SEP-NO HF), septic pigs with conventional hemofiltration (SEP-CHF), septic pigs with high-volume hemofiltration (SEP-HVHF). Data are median and 25th and 75th percentiles. + significant difference vs CON-NO HF ($p < 0.05$). Note different Y-scales in A-D.