THE ROLE OF ENDOTHELIUM-DERIVED VASOACTIVE MEDIATORS IN DIFFERENT TYPES OF CIRCULATORY DISORDERS

Ph.D. Thesis

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CONTENTS

LIST OF PAPERS RELATING TO THE SUBJECT OF THE DISSERTATION

I. Antal Wolfárd, József Kaszaki, Csaba Szabó, Zsolt Balogh, Sándor Nagy and Mihály Boros: EFFECTS OF SELECTIVE NITRIC OXIDE SYNTHASE INHIBITION IN HYPERDYNAMIC ENDOTOXEMIA IN DOGS. *Eur Surg Res* 31: 314-323, 1999.

II. Antal Wolfárd, József Kaszaki, Csaba Szabó, László Szalay, Sándor Nagy and Mihály Boros: PREVENTION OF EARLY MYOCARDIAL DEPRESSION IN HYPERDYNAMIC ENDOTOXEMIA IN DOGS. *Shock* (in press).

III. József Kaszaki, Antal Wolfárd, Mihály Boros, Lajos Baranyi, Hidechika Okada and Sándor Nagy: EFFECTS OF ANTIENDOTHELIN TREATMENT ON THE EARLY HEMODYNAMIC CHANGES IN HYPERDYNAMIC ENDOTOXEMIA. *Acta Chir Hung* 36: 152-153, 1997.

IV. Antal Wolfárd, Róbert Vangel, László Szalay, József Kaszaki, László Haulik, Ádám Balogh, Sándor Nagy and Mihály Boros: ENDOTHELIN-A RECEPTOR ANTAGONISM IMPROVES SMALL BOWEL GRAFT PERFUSION AND STRUCTURE FOLLOWING ISCHEMIA AND REPERFUSION. *Transplantation* (in press).

SUMMARY

Endothelium-derived vasoactive substances are important regulators of vascular tone and local blood flow. However, they can also play a role in the pathophysiology of different types of circulatory disorders, as compensatory or aggravating factors. In this respect, we have investigated the roles of the most important endothelium-derived vasodilator nitric oxide (NO) and vasoconstrictor endothelins (ETs), in (1) endotoxin-induced systemic and (2) ischemia-reperfusion-induced local circulatory disorders.

NO, formed by the isotypes of nitric oxide synthase (NOS), has been linked with the hypotension and myocardial depression in endotoxemia. We determined the activities of NOS isoenzymes in the heart and found an early activation of the endothelial NOS (eNOS). The inducible NOS (iNOS) activity increased later, but only after 8 h of endotoxemia. Although the participation of iNOS in early cardiac depression in our model is unlikely, it has been suggested that only NO overproduction by the iNOS is harmful in endotoxemia. We therefore compared the consequences of selective and non-selective NOS inhibition in a clinically relevant model of hyperdynamic endotoxemia. The non-selective NOS inhibitor N-ω-nitro L-arginine methyl ester restored the blood pressure and the depressed myocardial contractility, but reduced the cardiac output. The selective iNOS inhibitor S-methylisothiourea exerted only moderate hemodynamic effects during the initial 8 h of endotoxemia, which were probably related to a slight eNOS inhibition in a condition when iNOS is not yet activated. Mercaptoethylguanidine, a selective iNOS inhibitor and free radical scavenger, restored the myocardial performance and prolonged the compensatory hyperdynamic circulatory reaction. However, these effects of the compound are based upon the inhibition of granulocyte-derived free radical production and the attenuation of myocardial granulocyte deposition.

Sepsis is characterized by transitions between hyperdynamic, vasodilative and hypodynamic, vasoconstrictive phases. Elevated plasma levels of ETs in our hyperdynamic endotoxemic model suggest that these peptides modify the cardiovascular status in endotoxemia. Blockade of the vasoconstrictory ET-A receptors with ETR-p1/fl peptide resulted in a prolonged hyperdynamic response. Additionally, cardiac depression was antagonized by ET-A inhibition, demonstrating the role of the ETs in the mediation of endotoxin-induced early contractility failure. Thus, the increased cardiac output after the treatment can be a result of attenuated vasoconstriction and improved myocardial performance.

Vasoconstriction and hypoperfusion are the most important, non-immunologic complications after ischemia-reperfusion injury. In our dog model of small bowel transplantation, the typical, well-known pathologic consequences of reperfusion were observed, which include mesenteric hypoperfusion, activation and mucosal deposition of granulocytes and histological injury of the mucosa. Since all of these changes can be linked to the ETs, the ET-A antagonist ETR-p1/fl peptide was administered to protect the graft mucosa. The peptide prevented hypoperfusion, mucosal granulocyte accumulation and morphological damage after either systemic or local intragraft treatment, indicating the importance of endogenously released ETs and ET-A receptor activation in mesenteric ischemia-reperfusion states.

In conclusion, endothelium-derived vasoactive substances, together with other factors, are important in mediating the pathophysiologic events in various circulatory disorders. The inhibition of their production or effects may be beneficial, but the physiologic regulatory functions of these mediators should be preserved, in order to avoid aggravation of the injury.

1. INTRODUCTION

1.1. Circulatory disorders

The circulating blood transports oxygen and different nutritients to all organs and tissues of the body. Every factor which decreases the volume or the oxygen-transporting capacity of the blood, changes the normal distribution of the circulating blood or influences the blood supply of certain circulatory areas can result in inadequate oxygenation and nutritional problems as concerns the implicated tissues. When these states are categorized, a distinction may be made between generalized, systemic and localized circulatory disorders.

1.1.1. *Systemic circulatory disorders: circulatory shock states*

In systemic circulatory disorders, the whole circulatory system is affected by the initiating stimulus. This appears most frequently in different types of low cardiac output (CO), and high CO circulatory shock states. Low CO shock may have a peripheral origin (decreased preload after blood or plasma loss, e.g. hemorrhage or burning) or may be caused by the insufficient pump function of the myocardium (cardiogenic shock, e.g. myocardial infarction). In high CO states, peripheral vasodilation appears, and the afterload decreases, resulting in severe hypotension. This can occur after the liberation of large amounts of vasodilatory mediators (e.g. anaphylaxis) or in the hyperdynamic phase of septic/endotoxic shock.

1.1.2. *Clinical significance: sepsis, endotoxemia*

The development of sepsis is a frequent problem in intensive care units, among patients with severe injuries, in immunosuppression or in postoperative states. The mortality rate is high, especially when septic shock evolves, with severe peripheral vasodilation, myocardial depression and multiple organ failure (Carleton 1995, Ognibene 1997). Sepsis is a generalized reaction of the organism to different inflammatory stimuli. The septic inflammatory cascade can be initiated by the cell wall lipopolysaccharide (endotoxin) of Gram-negative bacteria, some cell components of Gram-positive bacteria, or other microorganisms. Macrophages and polymorphonuclear leukocytes produce inflammatory mediators (TNF-alfa and interleukins), which can further activate different cells (e.g. the cellular immune system or endothelial cells)

to produce mediators and express surface molecules. Some of the liberated inflammatory mediators have vasoactive effects. These can affect the myocardial function, acting together, synergistically or antagonistically, to produce the typical hemodynamic and metabolic events during sepsis (Parrillo 1993).

In the early phase of sepsis, a hyperdynamic circulatory reaction evolves, with an elevated or normal CO, a decreased peripheral resistance and hypotension, probably mediated by vasodilator mediators. Even if the CO is elevated, the myocardial contractility is depressed already during early sepsis. Metabolic abnormalities can also appear in this early phase. In the later phase of the process, the myocardial depression becomes more and more severe, peripheral vasoplegia appears, the ejection fraction decreases, resulting in a fall in CO and more severe hypotension, and septic shock develops. In this condition, a further decrease in organ perfusion and metabolic abnormalities can result in multiple organ failure. These conditions often lead to death, despite adequate antibacterial and vasopressor therapy and volume replacement.

1.1.3. *Local circulatory disorders: organ ischemia and ischemia-reperfusion*

In local circulatory disorders, the initiating stimulus affects only a part of the circulatory system. The pathologic events can remain localized, but can also lead to systemic circulatory reactions. Similarly, local circulatory disorders can also evolve as part of circulatory shock, like the mesenteric vasoconstriction in low CO states, as a result of the centralization of the circulation. Localized circulatory disorders are most frequently caused by the reduced or arrested blood flow to certain circulatory areas as a result of arterial or venous occlusion or vasoconstriction. The nutrition and the oxygenation of the tissues become insufficient, leading to tissue injury and functional abnormalities. In addition, after elimination of the inducing factor, the reflow of the blood to the previously ischemic tissues can further enhance the tissue injury.

Ischemia *per se* can lead to severe tissue injury, but it has been reported that the process of damage continues during the reperfusion phase (Granger 1988, Parks 1986). The typical pathologic events that appear during reperfusion are vasoconstriction, free radical generation, granulocyte activation and the accumulation of leukocytes in the reperfused tissues.

The main source of free radicals is the xanthine/xanthine oxidase system, but the oxidative burst of the activated granulocytes can also contribute to the free radical-mediated tissue injury (Granger 1988, Lojek 1997, Schoenberg 1991). Activated granulocytes, and endothelial cells in the reperfused area express adhesion molecules, resulting in enhanced leukocyte-endothelial interactions (Eppihimer 1997). The cells bind firmly to the endothelial surface, and in the next step migrate through the vessel wall toward the interstitial space of the organ. The liberated proteolytic enzymes of the extravasated granulocytes can also contribute to the tissue damage. Additionally, vasoconstriction and microvascular occlusion develop in the vascular bed of the reperfused tissue, inducing hypoperfusion and prolonging the ischemic time in certain areas of the involved organ (Boros 1995).

1.1.4. *Clinical significance: mesenteric ischemia-reperfusion, small bowel transplantation*

The mesenteric circulation utilizes a relatively large part of the CO, and thus the circulatory disturbances affecting this area often lead to severe local tissue injury and systemic hemodynamic reactions. The intestinal mucosa, which is a metabolically active, hypoxia-sensitive tissue, forms a barrier between the non-sterile intestinal lumen and the sterile circulation. Mucosal injury can therefore lead to bacterial and/or endotoxin translocation toward the blood, resulting in septic complications.

Mesenteric ischemia or ischemia-reperfusion appears in several pathologic conditions. Low CO states lead to the centralization of the circulation, and (as part of this process) mesenteric vasoconstriction evolves, leading to low-flow ischemia. Arterial (embolism, thrombosis and hernia incarceration) or venous occlusion in the mesenteric vascular bed also induce mesenteric ischemia. Reperfusion is possible only after elimination of these factors.

A large number of diseases can lead to an insufficient absorption capacity of the intestine. Two-thirds of all patients are infants with congenital malformations (intestinal atresia or microvillus inclusion bodies), causing malabsorption, but some other diseases (radiation enteritis, Gardner's syndrome and Crohn's disease) appearing in children and adults (Frezza 1996) may also cause this abnormality. The nutritional support of these patients must be managed with total parenteral nutrition. Transplantation of the small intestine now offers an optional treatment modality in these conditions (Kocoshis 1994). However, the application of

this therapy is limited by the high rate of immunologic and non-immunologic complications (Grant 1996, Reyes 1998). The intestine is very immunogenic and contains large amounts of lymphoid tissues; besides frequent rejection episodes, therefore, graft versus host disease often develops. Novel immunosuppressant strategies (tacrolymus instead of cyclosporine) have improved the results during the last few years, but the patient survival rate is still only about 50%, and the graft survival rate is also lower than after the transplantation of other organs (Grant 1996, Rossi 1996).

In addition to immunologic reactions, ischemia-reperfusion injury of the transplanted intestine may contribute to the development of early complications (Pirenne 1988), even if ischemia and reperfusion are well controlled during transplantation procedures (Cryer 1997). Most patients die of septic complications (Grant 1996), which can be induced by bacterial or endotoxin translocation, probably as a result of mucosal injury (Spada 1996, Browne 1991). Of course, rejection episodes can also contribute to the destruction of the mucosal barrier, but the role of ischemia-reperfusion injury is not negligible.

1.2. Endothelium-derived mediators in circulatory disorders

The endothelium is distributed throughout the body, and forms a selective permeability barrier between the circulating blood and the extravascular space. Additionally, endothelial cells are metabolically active and play an important role in the regulation of peripheral vascular tone, by producing different types of vasodilatory and vasoconstrictory mediators. In this way, the endothelium influences the blood pressure and regional blood flow. Similarly, endothelial cells are able to influence the coagulatory properties of the vessel wall and the process of leukocyte extravasation (Pearson 1991).

The most important endothelium-derived vasoactive mediators are the vasodilator nitric oxide (NO) and the vasoconstrictor endothelins (ETs). The basal release of these compounds by the endothelial cells and the balance between NO and the ETs contributes to the maintenance of physiologic vascular homeostasis.

The various circulatory disorders are frequently accompanied by vasodilation, vasoconstriction or changes in vascular reactivity in which the role of endothelium-derived

vasoactive substances is likely. These events can be a part of the pathologic cascade, or can play compensatory functions. In endotoxemia and sepsis, both vasodilation and vasoconstriction can develop during the different phases, suggesting the possible overproduction of NO and/or the ETs. In mesenteric ischemia-reperfusion, the sustained vasoconstriction plays a dominant role, indicating the participation of ETs in the pathomechanism, while the vasodilatory and antiadhesive effects of NO are directed toward the attenuation of reperfusion injury.

1.2.1. *Vasodilators: nitric oxide*

It was recognized in 1980 that endothelial cells produce a soluble, membrane-permeable molecule, which causes relaxation in isolated vascular rings. The molecule was termed "endothelium-derived relaxing factor", and was later identified as NO (Furchgott 1980, Moncada 1988). NO increases the formation of cyclic GMP, resulting in relaxation of the vascular smooth muscle cells, which causes vasodilation (Moncada 1991).

NO is formed by the enzyme nitric oxide synthase (NOS), through oxidation of the guanidino nitrogens of L-arginine. Three different isotypes of NOS have so far been recognized. The continuously active, Ca^{++}-dependent, constitutive forms of NOS are present in the endothelial cells (endothelial NOS, eNOS) and in the central nervous system (neuronal NOS, nNOS). The eNOS forms a small amount of NO that is involved in regulation of the peripheral vascular tone under physiologic conditions, inhibits the adhesion of leukocytes to the endothelial surface and exerts anticoagulatory effects by inhibiting platelet aggregation. The NO formed by the nNOS plays an important role as a neurotransmitter in the central nervous system (Moncada 1991).

In response to different inflammatory mediators or endotoxin (ETX), the third isoform of NOS, inducible NOS (iNOS) is activated in macrophages, leukocytes and other cell types, including cardiomyocytes (Bateson 1996, Schulz 1992, Szabó 1993). The activation of iNOS requires *de novo* protein synthesis, and the expression of iNOS therefore takes several hours after the initiating stimulus. iNOS produces a large amount of NO, which is involved in the defense mechanism of the organism, via its antibacterial killing effect. However, NO is also known to exert vasodilatory and cardiodepressive effects (Parratt 1998, Stein 1995, Sun

1998). It has therefore been suggested that the iNOS-induced overproduction of NO can mediate late septic vasodilation and vasoplegia, and can contribute to the development of severe myocardial depression, also characteristic of the hemodynamic pattern of septic shock (Parratt 1998, Thiemermann 1997).

The recognized hemodynamic effects of NO and the occurence of iNOS activation during sepsis raises the question of whether the inhibition of NO production by NOS inhibitors could have beneficial consequences in septic conditions.

1.2.2. *Nitric oxide synthase inhibition in sepsis*

Non-selective arginine analogs prevent sepsis- or ETX-induced hypotension, increase the peripheral vascular resistance and protect against endotoxemic myocardial depression (Herbertson 1996, Kaszaki 1996). In addition to these beneficial effects, however, several harmful consequences of the treatment were reported. The CO may fall critically after the administration of arginine analogs, while the blood flow to several organs decreases, resulting in tissue hypoperfusion (Henderson 1994, Spain 1994). Enhanced leukocyte extravasation and microthrombus formation have also been described (Jourdain 1997, Minnard 1994). These side-effects may be due to the non-selective NOS-inhibitory property of the compounds. The inhibition of eNOS diminishes eNOS-derived NO formation, and results in vasoregulatory disturbances.

The importance of the basal NO release by eNOS, and the fact that only the NO overproduction caused by iNOS induces harmful hemodynamic effects, call for NOS inhibitors which exert a more selective inhibitory effect toward the iNOS (Kilbourn 1997). A group of S-substituted isothiourea derivatives have been described as selective inhibitors of iNOS *in vitro* (Southan 1995). Two compounds of this group have been investigated widely. S-methylisothiourea (SMT) was found to protect against hypotension and led to an increased survival rate in septic rats (Szabó 1994). Similarly, it decreased the ETX-induced hepatic damage in rats, while the non-selective NOS inhibitor N-ω-nitro-L-arginine-methyl ester (L-NAME) enhanced the hepatic injury in the same model (Vos 1997). S-(2-aminoethyl) isothiourea also prevented hypotension in septic rats, without causing a pressor response in intact animals, suggesting that this compound does not inhibit eNOS (Southan 1995). S-(2-

aminoethyl) isothiourea spontaneously rearranges to mercaptoethylguanidine (MEG) in the circulation at neutral pH (Southan 1996). MEG restores cardiac efficiency after perfusion with proinflammatory cytokines *in vitro* (Panas 1998). Additional beneficial effects of MEG have also been elucidated. It is a strong inhibitor of cyclooxygenase, this action perhaps also contributing to its effects in endotoxemia, by decreasing the formation of cyclooxygenase-derived inflammatory products (Zingarelli 1997). MEG is also a scavenger of peroxynitrite free radicals, the products of the reaction of NO with superoxide (Szabó 1997).

It has been suggested that the inhibition of iNOS activity during sepsis with selective iNOS inhibitors can exert beneficial hemodynamic effects, without disturbing the normal vasoregulatory function of the eNOS-derived NO (Kilbourn 1997).

1.2.3. *Vasoconstrictors: endothelins*

The ETs are a family of 21-amino acid polypeptides, which are formed by the endothelial cells, and induce the slowly-evolving, but long-lasting constriction of vascular smooth muscle cells (Yanagisawa 1988). Three different isopeptides of the ET family are known at present. The peptides ET-1, ET-2 and ET-3 differ from each other principally in the affinity toward the specific ET receptors. The ETs are the most potent endogenous vasoconstrictor substances known to date, but they can also induce vasodilation (Rubanyi 1994). These multiple effects suggested the existence of different receptors, sometimes mediating different effects of the same ET.

Three different types of ET receptors have so far been recognized and isolated: the ET-A, ET-B and ET-C receptors. Additionally, the ET-B receptors can be subdivided into two subfamilies, the ET-B_1 and ET-B_2 receptors. The ET-A and ET-B_2 receptors are mainly localized on the surface of the vascular smooth muscle cells. Activation of these receptors causes constriction of the vascular smooth muscle cells, resulting in vasoconstriction, which is the most typical effect of the ETs (Rubanyi 1994). The ET-B_1 receptor subfamily is localized on the vascular endothelial cells, and mediates vasodilation, probably via the liberation of NO and prostacycline (Curzen 1995). The ETs have been reported to exert a positive inotropic effect *in vitro*, probably via the ET-B receptors (Beyer 1995), but *in vivo* the constriction of coronary vessels can counterbalance this effect (Naruse 1995).

1.2.4. *The role of endothelins in sepsis*

The physiologic function of the ETs is not very clear; they probably play a role in the regulation of peripheral vascular tone and blood flow as paracrine mediators. However, elevated plasma levels of ETs in different pathologic conditions suggest their important role in pathophysiologic circulatory reactions. Among others, elevated levels of circulating ETs have been reported in sepsis and in septic shock (Weizberg 1991). A number of experimental findings, using different ET receptor antagonists suggest that the ETs play a compensatory role against sepsis-induced hypotension (Mitaka 1998). On the other hand, ET-induced vasoconstriction can also cause deleterious effects by enhancing the pulmonary hypertension that occurs in sepsis (Mitaka 1998). It has been clearly established that the ETs, and ET-A receptor activation play a crucial role in hypodynamic sepsis (Szalay 1998). The effects of the ETs on myocardial contractility, and especially their possible role in sepsis-induced cardiac depression, have not been clarified yet.

1.2.5. *The role of endothelins in mesenteric ischemia-reperfusion*

Several observations suggest an important role for the potent vasoconstrictor ETs in the mediation of pathologic events during mesenteric ischemia-reperfusion. Elevated levels of circulating ETs have been observed under experimental conditions after superior mesenteric artery occlusion and reperfusion in pigs (Schlichting 1995). The sustained and significant vasoconstriction during the reperfusion phase also suggests the presence of a vasoconstrictor mediator or mediators (Boros 1995, Lock 1995). Additionally, the ETs can be linked to other ischemia-reperfusion-related pathologic changes. ET-1 enhances the free radical production of prestimulated granulocytes (Ishida 1990). Similarly, it has recently been reported that the administration of exogenous ET-1 induces leukocyte-endothelial interactions in the venules of the rat small intestine via an ET-A receptor-dependent mechanism (Boros 1998). These interactions comprise the first step in the process of granulocyte extravasation. The histologic damage to the intestinal mucosa after exogenous ET-1 infusion is similar to that observed after intestinal ischemia and reperfusion (Massberg 1998). The enhancement of leukocyte-endothelial interactions and the histological injury caused by the ETs can be prevented by the

ET-A receptor antagonist, BQ 610 or the ETR-p1/fl peptide, suggesting the participation of the vasoconstrictor ET-A receptors in ischemia-reperfusion (Boros 1998, Massberg 1998).

1.2.6. *Endothelin receptor antagonists. The ETR-p1/fl peptide*

A large number of different ET receptor antagonists have been developed. Some of these are non-selective, and inhibit both ET-A and ET-B receptors (bosentan). Other antagonists are selective toward either the ET-A receptor (BQ 610) or the ET-B receptor family (IRL 1038). The use of ET antagonists with different specificities can help in elucidation of the role of the ETs in physiologic regulation and in different pathologic conditions.

The ETR-p1/fl peptide is a newly developed, antisense homology box-derived peptide, with strong inhibitory potency against the ET-A receptor (Baranyi 1995). The sense-antisense interaction means that the peptide synthetized from the inactive DNA strand, is complementary to the peptide translated from the active DNA strand. These peptides are able to recognize and to bind to each other (Blalock 1984). A sense-antisense interaction exists not only between different peptides, but also between two parts of one peptide chain. These amino acid sequences are termed antisense homology boxes. There are a number of peptides in which the existence of antisense homology boxes has been recognized. The ETR-p1/fl peptide was developed by using the antisense homology boxes of the human ET-A receptor, with a special computer program. The peptide induced *in vitro* relaxation in vessel rings previously constricted with ET-1. The ET-A receptor antagonist BQ 123 exerted the same effect in this setting, demonstrating the ET-A receptor-inhibitory property of the ETR-p1/fl peptide (Baranyi 1998). Recent *in vivo* results support the ET-A receptor-inhibitory potency of this peptide. It is able to attenuate ET-induced renal vasoconstriction (Wu 1997) and protects against the ET-A receptor-mediated circulatory effects of exogenous ET-1 (Szalay 1998).

1.3. Aims of the dissertation

We have investigated hyperdynamic endotoxemia as an example of systemic circulatory failure, and small bowel transplantation-reperfusion as an example of localized circulatory failure. These circulatory disorders can be linked with each other at several points. Low-flow ischemia and reperfusion of the mesenteric vascular bed can appear as a consequence of the shift between vasodilative and vasoconstrictive phases during sepsis. On the other hand, sepsis is the most frequent complication following small bowel transplantation and mesenteric ischemia-reperfusion, as a consequence of mucosal barrier destruction and bacterial translocation.

In Studies I and II we investigated the role of the most important endothelium-derived vasodilator, NO in ETX-induced systemic circulatory failure. In Studies III and IV, the role of the vasoconstrictor ETs was determined in ETX-induced systemic and transplantation-reperfusion-induced local, mesenteric circulatory disorders, respectively.

In **Study I,** we compared the effects of selective and non-selective NOS inhibition on the peripheral hemodynamics, myocardial contractility and cardiac constitutive, eNOS and iNOS activities in hyperdynamic endotoxemia in dogs.

In **Study II,** the effects of the selective iNOS inhibitor and free radical scavenger MEG were studied on the peripheral hemodynamic changes, myocardial contractility and cardiac eNOS and iNOS activities in hyperdynamic endotoxemia. The activation and the myocardial accumulation of granulocytes were also determined.

In **Study III,** we investigated the consequences of ET-A receptor inhibition in hyperdynamic endotoxemia on the peripheral hemodynamic parameters and myocardial contractility in a dog model.

In **Study IV,** we studied the role of endogenous ETs and the effects of ET-A receptor blockade on the mesenteric hemodynamic parameters, the activation and mucosal accumulation of granulocytes and the morphological changes of the intestinal mucosa following small bowel autotransplantation in dogs.

2. MATERIALS AND METHODS

2.1. Surgical preparation

2.1.1. *Studies I and II - Instrumentation for the measurement of myocardial contractility and peripheral hemodynamics*

The surgical interventions were identical in Studies I and II. The experiments were performed on a total of 59 healthy mongrel dogs. The average weight was 15±3 kg. Anesthesia was induced with sodium pentobarbital (30 mg/kg i.v.). Supplementary small doses (2 mg/kg/30 min i.v.) were administered during the experiments. After intubation of the trachea, the animals were ventilated with room air, using a Harvard respirator (Harvard Apparatus, South Natick, MA, U.S.A.). The left femoral artery and vein were cannulated for the measurement of mean arterial pressure (MAP) and the administration of drugs and fluids, respectively. The animals received an infusion of 7 ml/kg/h of Ringer's lactate during the experiments. A Swan-Ganz thermodilution catheter (Corodyn TD-E-N, 5011-110-7Fr; Braun Melsungen AG, Melsungen, Germany) was positioned into the pulmonary artery via the right femoral vein to measure CO. An inflatable balloon-catheter (Foley catheter, 14Fr, Kendall Company Ltd., Basingstoke, U.K.) was introduced into the inferior caval vein via the left jugular vein. A catheter-tip micromanometer (Millar Instruments Inc., Houston, TX, U.S.A.) was introduced into the left ventricle through the left internal carotid artery, for monitoring of the left ventricular pressure (LVP). A left thoracotomy was performed at the sixth intercostal space, the pericardium was opened, and a control myocardial biopsy sample was taken. A pair of ultrasonic dimension crystals (3 MHz, ID-4, Custom Transducers, Poway, CA, U.S.A.) were sutured onto the anterior and posterior walls of the left ventricle, opposite each other, using an atraumatic surgical technique. The correct alignment of the crystals was confirmed with an oscilloscope (EMG-4572, Orion, Budapest, Hungary). The thoracic cavity was revised, and the chest wall was closed in four layers. The air was removed from the thorax, and the animals were then breathing spontaneously. The body temperature was maintained at 37 °C with a homeothermic blanket. At the end of the experiments, a myocardial tissue biopsy sample was taken from the left ventricle and the animals were killed with an overdose of pentobarbital.

2.1.2. *Study III*

The experiments were performed on 26 healthy, mongrel dogs. The average weight was 15±3 kg. In this study, the instrumentation for the measurement of myocardial contractility was identical to that described previously, except that we did not perform myocardial tissue biopsy.

2.1.3. *Study IV - Small bowel autotransplantation*

Four separate groups of experiments were performed on a total of 29 mongrel dogs (weighing 25.4±4 kg) under sodium pentobarbital anesthesia (30 mg/kg i.v.). The animals were deprived of food, but not water, for 24 h prior to the experiments. The trachea was intubated to facilitate respiration and the body temperature was maintained at 37 °C with a homeothermic blanket. Using aseptic techniques, the right femoral artery and vein were cannulated for the measurement of MAP and for fluid and drug administration, respectively. All animals received a continuous infusion of Ringer's lactate at a rate of 10 ml/kg/h.

In Groups 1, 2 and 3, orthotopic small bowel transplantation (SBTX) was performed. Following an upper transverse laparotomy, the spleen was removed, and the root of the superior mesenteric artery (SMA) was dissected free. An ultrasonic flow-probe (Transonic Systems Inc., Ithaca, NY, U.S.A.) was placed around the exposed vessel to measure mesenteric blood flow. A branch of the ileocolic artery and a tributary of the ileal vein supplying the terminal part of the ileum were cannulated with 2-F polyethylene catheters to measure the mesenteric arterial and venous pressures, respectively. The mesentery was divided between ligations at the distal (ileo-colic) and proximal (duodeno-jejunal) part of the small bowel, the intestinal wall was transected, and a control tissue biopsy was taken from the ileal part of the small bowel. The SMA was then cannulated with a 7-F polyethylene catheter and the intestinal graft was perfused with 500 ml of chilled (4 °C) heparinized Ringer's lactate at 100 water-cm perfusion pressure. The cold ischemia time was 60 min. The graft was stored on ice until the vessels were reanastomized end-to-end with 6/0 running sutures (non-absorbable polypropylene). The animals were observed for 4 h after the revascularization, an ileal section of the small intestine was then taken and the animals were killed with an overdose of pentobarbital.

Group 4 served as sham-operated controls to exclude hemodynamic changes related solely to surgery and to the time elapsed under anesthesia. In these experiments, the animals were infused continuously with Ringer's lactate and received the vehicle for ETR-p1/fl (saline) at matching time points after surgery. There was no significant difference between the groups as concerns the duration of the surgical preparation and the cold ischemia time.

2.2. Hemodynamic measurements

2.2.1. *Blood pressures and heart rate*

MAP, central venous pressure (CVP) and pulmonary artery pressure (in Studies I, II and III) and also superior mesenteric arterial (SMAP) and venous (SMVP) pressure (in Study IV) were monitored with Statham P23Db transducers and recorded with a computerized data-acquisition system (Experimetria Ltd., Budapest, Hungary). Heart rate was measured via conventional electrocardiograms.

2.2.2. *Cardiac output and peripheral resistance (in Studies I, II and III)*

CO was determined by thermodilution, using a Cardiostar CO-100 computer (Experimetria Ltd., Budapest, Hungary). Five ml of physiologic saline (2-5 °C) was used as indicator. Measurements were made in duplicate at each sampling time point, and the average value of the two measurements was calculated. Total peripheral vascular resistance (TPR) was calculated via the standard formula (MAP-CVP)/CO.

2.2.3. *Superior mesenteric artery flow and mesenteric vascular resistance (Study IV)*

Superior mesenteric artery blood flow (SMAF) was monitored continuously with an ultrasonic flow-meter (Transonic Systems Inc., Ithaca, NY, U.S.A). The flow-probe was placed around the root of the SMA. Mesenteric vascular resistance (MVR) was calculated by using the standard formula (SMAP-SMVP)/SMAF. Hemodynamic data were recorded with a computerized data-acquisition system. Arterial blood gases were measured with a blood gas analyser (Radiometer, Copenhagen, Denmark).

2.2.4. *Myocardial contractility (in Studies I, II and III)*

The ultrasonic dimension crystals were connected to a sonomicrometer (Triton Technology, Inc., San Diego, CA, U.S.A.) and the signal of the ventricular diameter was monitored with an oscilloscope. The LVP and diameter signals were digitized with an analog-to-digital converter of an IBM-compatible personal computer. The end-systolic elastance, as the parameter of the left ventricular myocardial contractility, was estimated from the slope of the end-systolic pressure-diameter relationship (Goldfarb 1982, Sagawa 1981) with a computer program developed by our group. The inferior vena cava was briefly occluded by a balloon catheter, and pressure-diameter loops were obtained for 8 s. The end-systolic points of the loops (which can be fitted to a sigmoid curve) were recorded and displayed on the monitor screen. The linear part of the curve was selected on the basis of the lowest variance, and a straight line was fitted to the selected points. The computer program calculated the myocardial contractility as the slope of the end-systolic pressure - diameter relationship. The intercept of the slope with the x (the diameter) axis, and the variance of fitting were determined. The calculation was based on a minimum of eight cardiac cycles.

2.3. Biochemical measurements

2.3.1. *Myocardial nitric oxide synthase activity (in Studies I and II)*

NO formation in cardiac tissues was measured by the conversion of [^{3}H]L-citrulline from [^{3}H]L-arginine according to the method of Szabó et al. (Szabó 1993). Briefly, heart biopsies kept on ice were homogenized in phosphate buffer pH 7.4 containing 50 mM tris-(hydroxymethyl)aminomethane (Tris), HCl (Reanal, Budapest, Hungary), 0.1 mM ethylenediamine-tetraacetic acid (EDTA, Serva Feinbiochemica GmbH, Heidelberg, Germany), 0.5 mM dithiotreitol, 1 mM phenylmethylsulfonyl fluoride and 10 μg/ml of soybean trypsin inhibitor. The homogenate was centrifuged at 4 °C for 20 min at 13 000 g and the supernatant was loaded into centrifugal concentrator tubes (Amicon Centricon-100; 100 000 MW cut-off ultrafilter). The tubes were centrifuged at 1000 g for 150 min and the concentrated supernatant was washed out from the ultrafilter with 250 μl of homogenizing buffer. The samples were incubated with a cation-exchange resin (DOWEX AG 50W-X8, Na^+ form) for 5 min to

deplete endogenous L-arginine. The resin was separated by centrifugation (1500 g for 10 min) and the supernatant containing the enzyme was assayed for NOS activity.

For the Ca^{++}-dependent NOS (eNOS) activity, 50 µl of enzyme extract and 100 µl of reaction mixture (pH 7.4, containing 50 mM Tris HCl buffer, 1 mM NADPH, 10 µM tetrahydrobiopterin, 1.5 mM $CaCl_2$, 100 U/ml calmodulin and 0.5 µCi of [^{3}H]L-arginine (Amersham U.K., specific activity 63 Ci/mmol) were incubated together for 60 min at 37 °C. The reaction was stopped by the addition of 1 ml ice-cold HEPES buffer (pH 5.5) containing 2 mM EGTA and 2 mM EDTA. Measurements were performed with boiled enzyme and with the NOS inhibitor N-ω-nitro-L-arginine (3.2 mM) to determine the extent of [^{3}H]L-citrulline formation independent of the NOS activity. Ca^{++}-independent NOS activity (iNOS) was measured without Ca-calmodulin and with EGTA (8 mM).

1 ml of the reaction mixture was applied to DOWEX cation-exchange resin (AG 50W-X8, Na^+ form) and eluted with 2 ml of distilled water. The eluted [^{3}H]L-citrulline activity was measured with a scintillation counter (Tri-Carb Liquid Scintillation Analyzer 2100TR/2300TR, Packard Instrument Co, Meriden, CT, U.S.A.). Protein contents of samples were determined by the method of Lowry et al. (Lowry 1951).

2.3.2. *Granulocyte free radical production capacity and tissue myeloperoxidase activity (in Study II and IV)*

Granulocytes were separated from the systemic venous blood in Study II, or from the effluent venous blood of the intestine in Study IV by the method of Guarnieri et al. (Guarnieri 1990). Following dextran sedimentation and hypotonic lysis (to remove contaminating erythrocytes), Ficoll-Hipaque gradient centrifugation was performed. The cells (95% neutrophil granulocytes) were washed twice in phosphate-buffered saline with 5 mM glucose at pH 7.4. The granulocytes (1.5 x 10^6 cells/ml) were incubated at 37 °C for 3 min in the presence of 0.1 mM ferricytochrome c in phosphate buffer, and the generation of superoxide (O_2^-) was detected at 550 nm with a UV-1601 spectrophotometer (Shimadzu, Japan). Spontaneous radical production and the phorbol myristate acetate (PMA) (0.1 mg/ml)-stimulated activity of the granulocytes were determined. The release of O_2^- into the reaction

mixture was calculated from the linear portion of the cytochrome c reduction plot, using a molar absorption coefficient of $29.5x10^3$/M/cm.

Myeloperoxidase (MPO) activity, as a marker of tissue leukocyte infiltration, was measured on cardiac biopsy samples in Study II and on mucosal biopsies in Study IV. The MPO activity was determined according to the method of Kuebler et al. (Kuebler 1996).

2.3.3. *In vitro granulocyte activation (in Study II)*

Granulocytes were separated from healthy, mongrel dogs according to the method of Guarnieri et al. (Guarnieri 1990). 4.5 million cells were placed into 6 ml of glucose-containing Dulbecco-PBS solution in Petri dishes. Luminol (100 μM) was added to each plate. Before activation, either MEG (1 μM, 10 μM, 100 μM or 1 mM) or vehicle (saline) was added to the mixture. The free radical production of the granulocytes was determined by measuring the luminol-enhanced chemiluminescence using a computer-controlled luminometer (Mikro GM, Szeged, Hungary). After baseline chemiluminescence recording, PMA (0.1 μM) was added to the cells and the maximal activation (counts per second - cps) was recorded. The effect of MEG on the luminol-enhanced chemiluminescence of the granulocytes was expressed as a percentage of the activation (cps) in the MEG-treated samples vs. that in non-treated control samples.

2.3.4. *Plasma levels of endothelins (in Study III)*

The plasma levels of circulating ETs were measured by radioimmunoassay. Blood samples were collected in tubes containing ice-cold heparin (750 IU in 150 μl of saline), and aprotinin (500 KIU/ml). The blood was centrifuged immediately (1200 g at 4 °C for 10 min), and plasma was stored below -20 °C until used. The ETs were extracted from the plasma by using Amersham's Amprep 500 mg C2 columns (code RPN 1913). The mini-columns were previously equilibrated with 2 ml of ethanol and 2 ml of water. The plasma was acidified with 0.5 ml of 2 M HCl and after centrifugation the sample was loaded onto the column. The eluent was collected in 2 ml of methanol-water (80% methanol in water containing 0.1% trifluoroacetic acid). The solvent was evaporated under a N_2 atmosphere and reconstituted with 250 μl of assay buffer (0.02 M borate, pH 7.4, containing sodium azide). Plasma ET

levels were measured with a commercial ET-1,2 (^{125}I) RIA kit (Amersham, UK, code number RPA 535 and RPA 545). Radioactivity was measured in a gamma counter for 2 min.

2.4. Light microscopy (in Study IV)

Intestinal tissue samples for light microscopy were fixed in 6% buffered formalin, embedded in paraffin, sectioned (6 μm) and stained with hematoxylin and eosin. Mucosal damage was assessed on the standard scale of Chiu et al. (Chiu 1970). The grading was performed on coded sections with the following criteria: grade 0, normal mucosa; grade 1, development of subepithelial Gruenhagen's space at the tip of the villus; grade 2, extension of the space with moderate epithelial lifting; grade 3, massive epithelial lifting with a few denuded villi; grade 4, denuded villi with exposed capillaries; and grade 5, disintegration of the lamina propria, ulceration and hemorrhage. Five randomly selected non-overlapping fields per section were inspected and the median grade of injury was then calculated.

2.5. Experimental protocol

2.5.1. *Study I*

Surgery was followed by a recovery period for cardiovascular stabilization and by a 30-min control period to determine baseline variables. Four groups were studied: Group 1 (n=10) comprised sham-operated controls, while groups 2 (n=7), 3 (n=7) and 4 (n=7) received a 2-h infusion of a total dose of 5.3 μg/kg *E. coli* O55:B5 ETX, (Sigma Chemicals Company, St. Louis, U.S.A.) in 6 ml of saline. The initial infusion rate was 5 mg/kg/h for 5 min, then 2.5 mg/kg/h for 115 min. Group 2 served as endotoxemic control (ETX group). In groups 3 and 4, NOS-inhibitor treatment was started after 45 min of endotoxemia. Group 3 received L-NAME, (Sigma Chemicals Company, St. Louis, U.S.A.) in a dose of 12.5 mg/kg in a 20-min infusion (ETX+L-NAME group). Group 4 was treated with SMT in a dose of 2 mg/kg (ETX+SMT group). The animals were observed for 8 h after ETX infusion. The volume load was identical in all groups studied.

2.5.2. *Study II*

Surgery was followed by a recovery period for cardiovascular stabilization and a 30-min control period. The animals were randomly allotted to one or other of two groups. Group 1 (n=14) served as endotoxemic control and received a 2-h infusion of *E. coli* ETX in 6 ml of saline. The protocol of endotoxemia-induction was the same as in Study I (ETX group). In group 2 (n=14, ETX+MEG group), MEG treatment (2 mg/kg i.v.) was started 45 min after endotoxemia induction. Seven animals in both experimental groups were sacrificed after 4 h of endotoxemia to obtain myocardial biopsy samples for cardiac NOS and MPO activity measurements. The remaining animals were observed for 8 h after the onset of ETX treatment. At the end of the observation period, the chest was opened and a myocardial biopsy sample was taken for NOS and MPO activity measurements.

2.5.3. *Study III*

After surgery, a recovery time was allowed, which was followed by a 30-min control period. The animals were divided into 4 groups. Group 1 (n=6) comprised sham-operated controls (Sham-operated group). In group 2 (n=6), the ET-A receptor antagonist ETR-p1/fl peptide (VLNLCALSVDRYRAVASWRVI, Kurabo Ltd., Osaka, Japan) was infused in a dose of 0.1 mg/kg (40 nmol/kg) to non-endotoxemic animals after the 30th min of the experiments (Sham-operated+ETR-p1/fl group). In groups 3 (n=7) and 4 (n=7), endotoxemia was induced in the same way as in Studies I and II. The animals in group 4 received an infusion of ETR-p1/fl peptide in a dose of 0.1 mg/kg after the 30th min of endotoxemia (ETX group and ETX+ETR-p1/fl group, respectively). The hemodynamic parameters and plasma ET levels were monitored for 4 h. The volume load was identical in all groups.

2.5.4. *Study IV*

The animals were randomized to one or other of the following 4 groups. In groups 1, 2 and 3, orthotopic SBTX was performed, as described previously. Group 1 (n=9) served as transplanted control (SBTX group). The animals in group 2 (n=7) were treated with ETR-p1/fl peptide in a dose of 0.1 mg/kg intravenously (i.v.) at the onset of reperfusion (SBTX+ETR-p1/fl i.v.). In group 3 (n=6), the harvested intestine was perfused with the same dose of ETR-

p1/fl peptide during the cold ischemic time (intragraft treatment, SBTX+ETR-p1/fl i.g.). Immediately before reanastomosis, the graft in group 3 was flushed again with 100 ml of chilled Ringer's lactate. The cold ischemia time was identical in all transplanted groups (60 min). The animals in group 4 (n=7) served as sham-operated controls (Sham-operated group). Control hemodynamic data were recorded before dissection of the mesenteric vessels (-120 min) and before harvesting of the graft (-60 min). The hemodynamic changes were monitored continuously during the 60-min ischemia (from -59 min to -1 min) and after the start of reperfusion (1 min). Control tissue and blood samples were taken at -60 min. Samples from the effluent venous blood of the intestinal graft were collected at 60-min intervals. After a 4-h reperfusion, tissue samples were taken from the ileal part of the transplanted intestine in order to determine mucosal MPO activity and to make histological sections. The volume load was identical in all groups studied.

2.6. Statistical analysis

The statistical analysis of our data was performed with non-parametric statistical methods, using a statistical software package (Jandel Scientific, Erkrath, Germany). Statistical differences within the groups, compared to the baseline values, were investigated by using Friedmann and Dunn's method, or the Wilcoxon signed rank test, depending on the number of sampling points. Intergroup comparisons were performed with Kruskal-Wallis and Dunn's method (4 different groups) or with the Mann-Whitney U test (2 groups in Study II). $p<0.05$ was considered a significant difference. In the Figures and Tables, median values, and 25th and 75th percentiles are given. Box plot figures demonstrate the median, and the 10th (lower whisker), 25th (bottom of the box), 75th (top of the box) and 90th (upper whisker) percentiles.

3. RESULTS

3.1. Study I - Selective and non-selective nitric oxide synthase inhibition in endotoxemia

There were no significant hemodynamic changes relative to the baseline values in the sham-operated group during the 8-h experimental period. Significant differences in the Figures are shown by the following symbols: * $p<0.05$ within the groups, X $p<0.05$ Sham-operated vs. ETX, # $p<0.05$ ETX vs. ETX+L-NAME or SMT groups, respectively.

A typical hyperdynamic circulatory reaction was observed in the ETX group, characterized by an increase in CO and a significant decrease in TPR for 2 h. Thereafter, the values stabilized near the control levels. ETX infusion induced a prompt decrease in MAP. After the end of ETX infusion, MAP increased toward the control level, but hypotension persisted until the end of the observation (Figure I/1). Non-selective NOS inhibition with L-NAME induced a significant decrease in CO and an increase in TPR, which lasted until the end of the experiments. L-NAME treatment immediately restored MAP, while treatment with the selective iNOS inhibitor SMT resulted in only a moderate increase (Figure I/1). SMT induced merely a transient decrease in CO and an increase in TPR, as compared with the ETX group (Figures I/2 and I/3).

Figure I/1 Mean arterial pressure

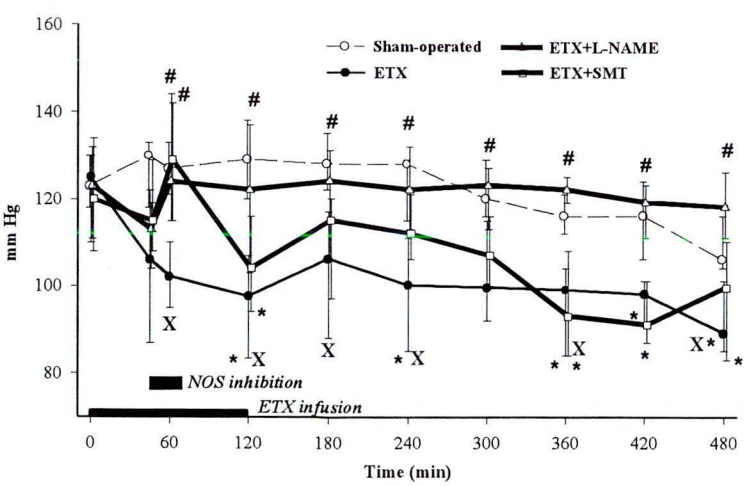


Figure I/2 Cardiac output

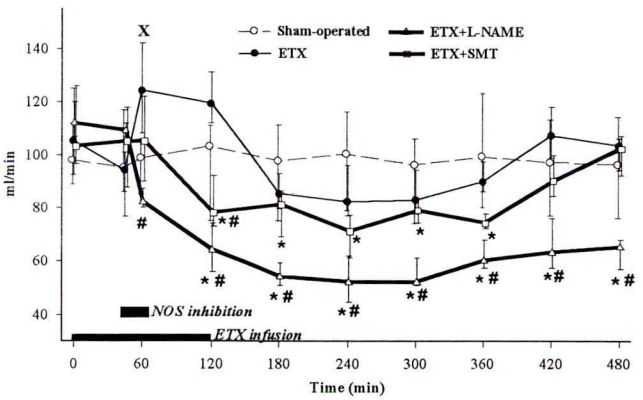
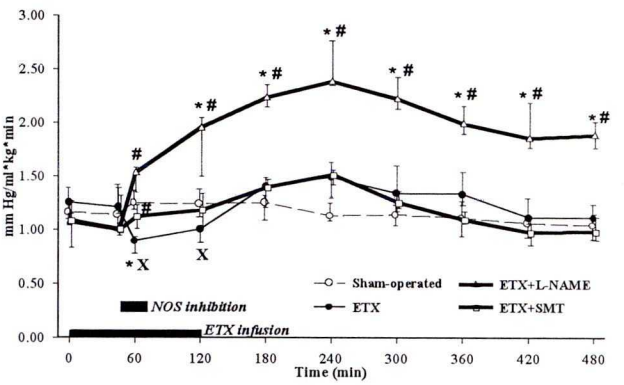
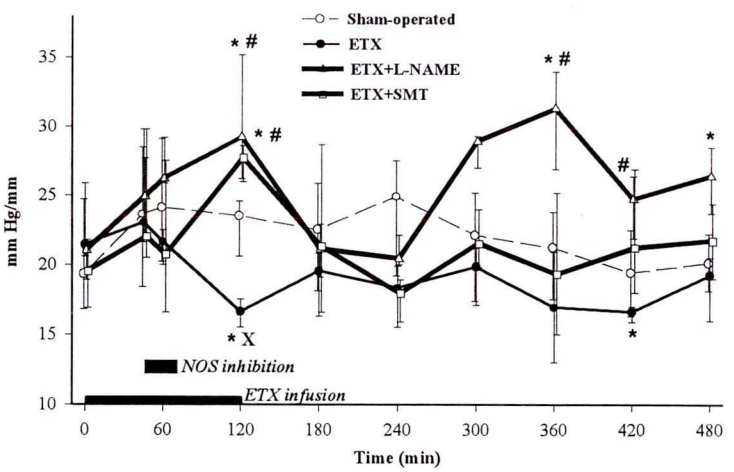


Figure I/3 Total peripheral resistance



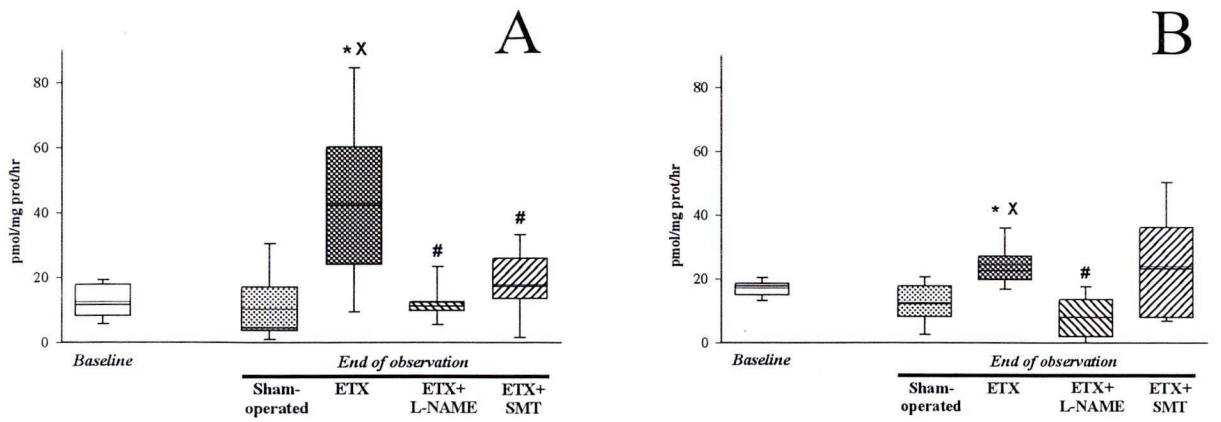
The left ventricular contractility was decreased after the low-dose ETX infusion. L-NAME treatment caused a biphasic, long-lasting increase in myocardial contractility. SMT increased myocardial contractility only in the first hour after infusion (Figure I/4).

Figure I/4 Left ventricular contractility



The activities of myocardial eNOS and iNOS were significantly increased after 8 h of endotoxemia, as compared with the values for the sham-operated group or with those measured in the control biopsy samples from ETX-treated animals. L-NAME significantly decreased the activities of both isoenzymes. SMT treatment significantly inhibited iNOS without influencing the myocardial eNOS activity (Figure I/5).

Figure I/5 Myocardial iNOS (A) and eNOS (B) activities



3.2. Study II - Effects of mercaptoethylguanidine in hyperdynamic endotoxemia

Significant differences in the Figures and in the Table are shown by the following symbols: * $p<0.05$ within the groups, # $p<0.05$ between the groups.

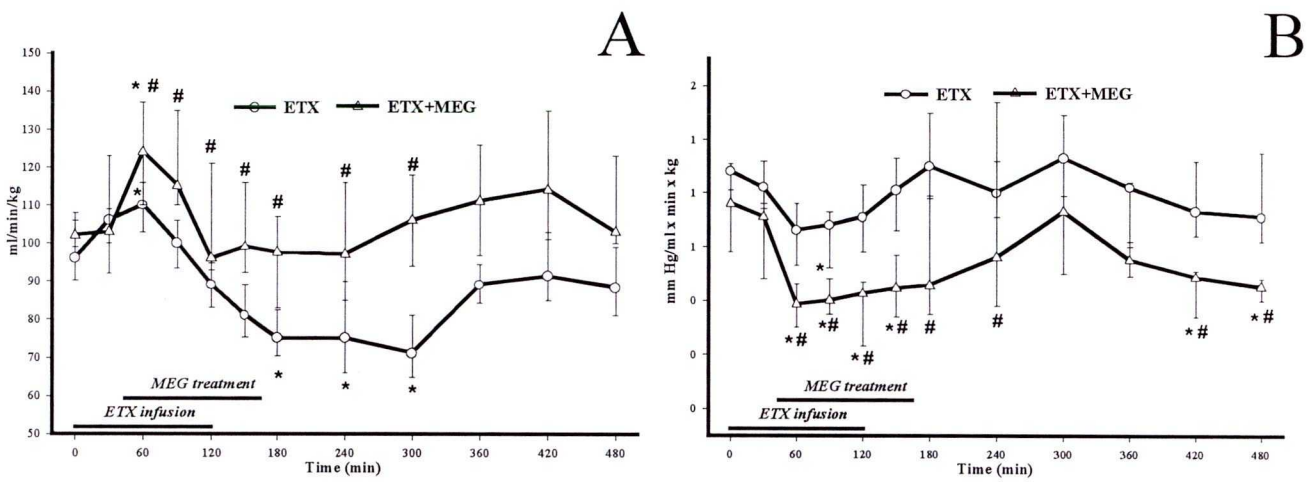
ETX induced a significant, 30% decrease in MAP in both groups. After the ETX infusion had been stopped, MAP increased toward the control level, but the hypotension persisted until the end of the 8-h observation period. The MEG infusion did not significantly influence the changes in MAP (Table II/1).

Table II/1 Mean arterial pressure

Time (min)	Mean arterial pressure (mm Hg)	
	ETX	ETX+MEG
0	122 (121, 132)	122 (119, 135)
30	119 (115, 125)	121 (115, 131)
60	111 (106, 113) *	116 (96.9, 127)
90	104 (96.8, 119)	102 (93.5, 120) *
120	102 (76.2, 110) *	88.7 (86, 94.8) *
150	90.1 (75, 109) *	98 (94, 103) *
180	99.7 (89.8, 111) *	111 (99.1, 116)
240	99.3 (91.3, 114) *	106 (97.6, 112) *
300	95.7 (85.5, 115)	101 (94.8, 114)
360	111 (101, 118)	98 (90, 112) *
420	102 (100, 108) *	91 (89.3, 105) *
480	103 (100, 108) *	91 (87, 100) *

During the first 2 h of endotoxemia, a hyperdynamic circulatory reaction evolved, characterized by a significant increase in CO and a reduction in TPR. Thereafter, CO decreased significantly, but both parameters stabilized by the end of the endotoxemia. The MEG treatment stabilized CO on a significantly higher level, and TPR on a lower level, than the values in the ETX group before the end of the observation period (Figures II/1/A and II/1/B).

Figure II/1 Cardiac output (A) and total peripheral resistance (B)



The ETX infusion caused a significant myocardial depression, which started in the 3rd h of endotoxemia. MEG prevented the negative inotropic effect of ETX; in the ETX+MEG group, the contractility values were significantly higher than those in the ETX group (Figure II/2).

Figure II/2 Left ventricular contractility

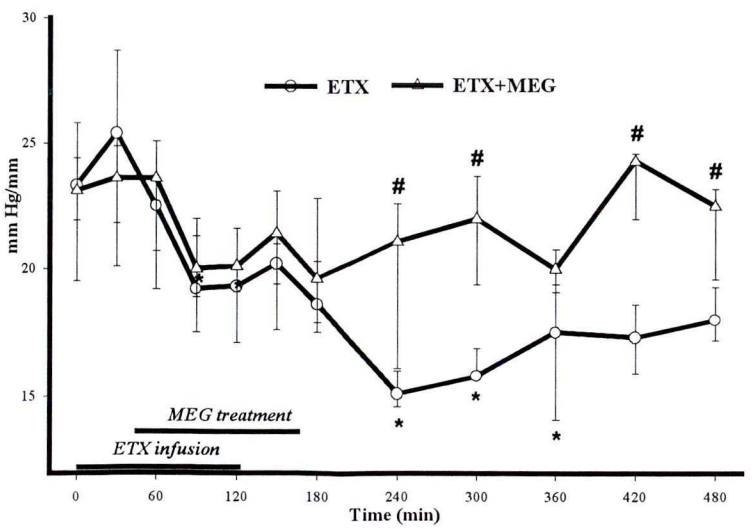
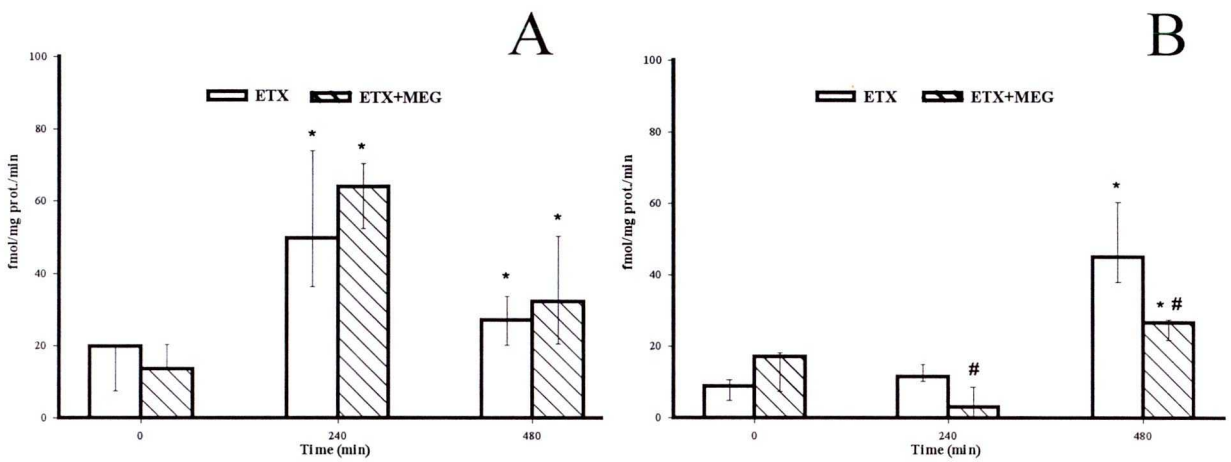


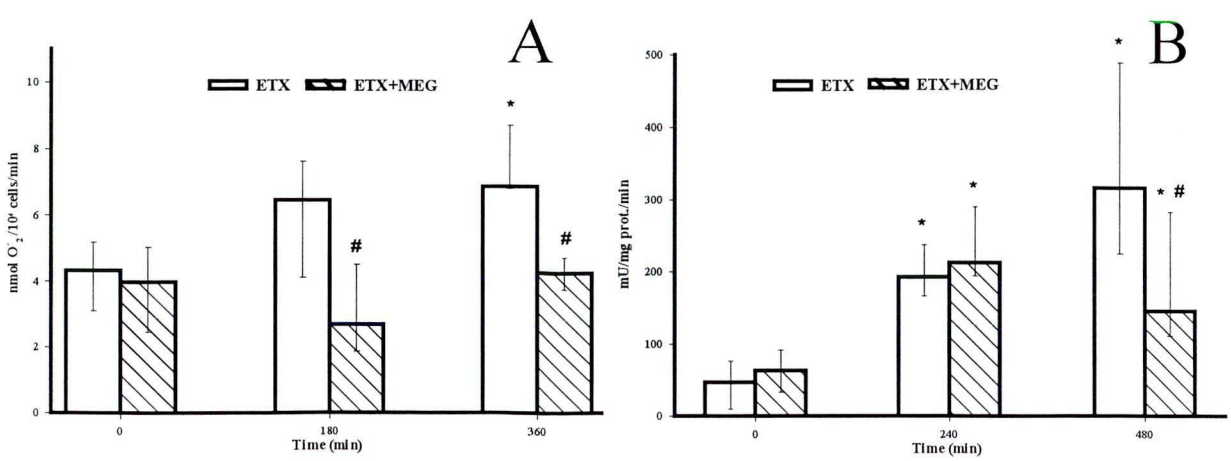
Figure II/3 depicts the changes in the myocardial eNOS and iNOS activities after 4 and 8 h of endotoxemia. The eNOS activity was significantly increased 4 h after endotoxemia induction, then decreased up to the end of the experiments, but remained higher than the baseline values. The iNOS activity was not increased significantly 4 h after the start of ETX infusion, but only after 8 h of endotoxemia. MEG selectively influenced the activity of myocardial iNOS. In the MEG-treated group, the values were significantly lower than those in the ETX group 4 and 8 h after endotoxemia induction. MEG did not influence eNOS activity.

Figure II/3 Myocardial eNOS (A) and iNOS (B) activities



The free radical-producing capacity of the granulocytes was gradually, but significantly increased during endotoxemia. The MEG treatment stabilized this parameter at the control level (Figure II/4/A). The myocardial MPO increased after ETX treatment. The granulocyte accumulation within the myocardium was increased in the MEG-treated group, but the MPO activity was significantly lower than in the control endotoxemic group (Figure II/4/B).

Figure II/4 Free radical production (A) and myocardial MPO activity (B)



In vitro granulocyte activation

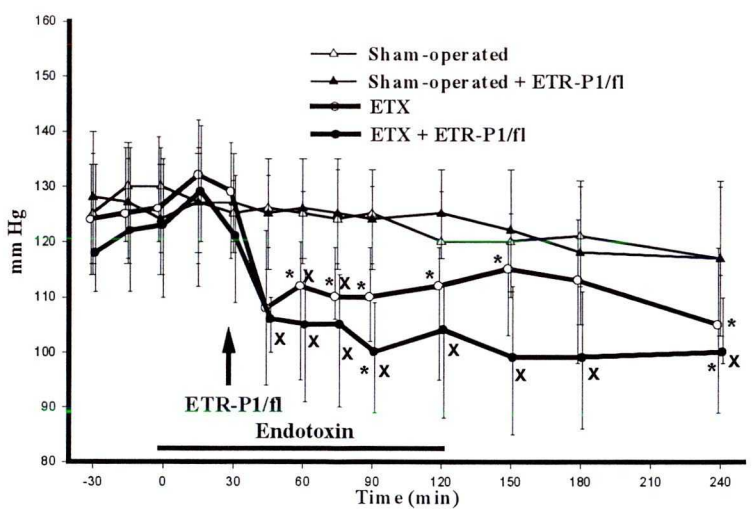
MEG dose-dependently decreased the luminol-enhanced chemiluminescence of the isolated granulocytes. The effective doses of the compound ranged between 10 μM and 1 mM. 10 μM, 100 μM and 1 mM MEG induced 18.5%, 42.9% and 63.2% decreases, respectively.

3.3. Study III - Endothelin-A receptor blockade in hyperdynamic endotoxemia

Significant differences in the Figures are shown by the following symbols: * $p<0.05$ within the groups, X $p<0.05$ compared with the Sham-operated group, # $p<0.05$ ETX vs. ETX+ETR-p1/fl.

There were no significant differences in the sham-operated group or in the group receiving ETR-p1/fl peptide alone, in either of the examined parameters. In the endotoxemic groups, ETX infusion induced a significant hypotension. After the infusion, MAP increased toward the baseline level, but the hypotension remained significant until the end of the experiments (Figure III/1).

Figure III/1 Mean arterial pressure



ETX induced a hyperdynamic circulatory reaction, characterized by an increase in CO and a significant decrease in TPR. After the infusion, these changes subsided in the ETX group, while the blockade of ET-A receptors with ETR-p1/fl peptide stabilized CO on a higher level, and TPR on a lower level until the end of the observation (Figures III/2 and III/3).

Figure III/2 Cardiac output

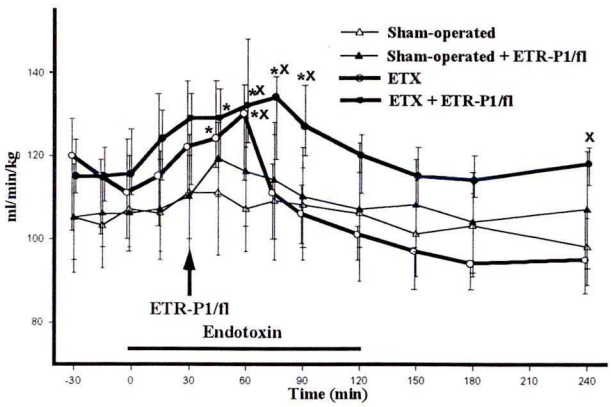
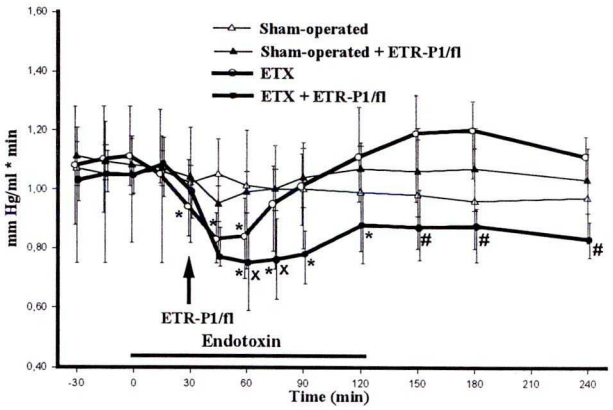


Figure III/3 Total peripheral resistance



In response to ETX a significant myocardial depression evolved after the 60th min of endotoxemia. ETR-p1/fl treatment prevented the ETX-induced cardiac depression (Figure III/4).

A long-lasting, 2.5-fold increase in plasma ET levels was observed in both endotoxemic groups. The treatment with ETR-p1/fl peptide delayed, but did not prevent the rise in plasma ET level (Figure III/5).

Figure III/4 Myocardial contractility

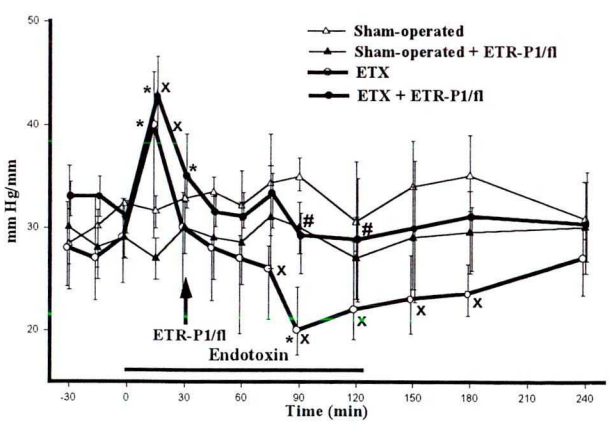
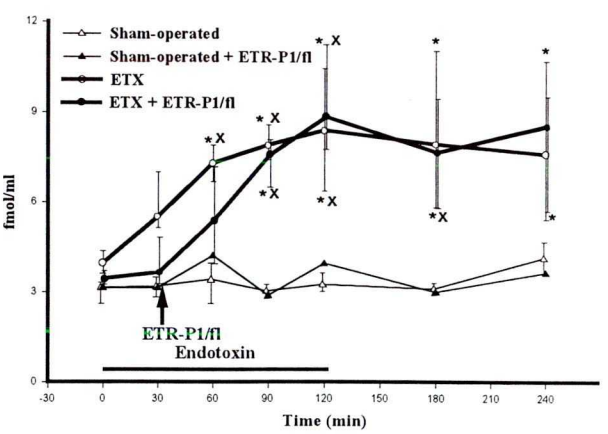


Figure III/5 Plasma endothelin levels



3.4. Study IV - Endothelin-A receptor inhibition in small bowel autotransplantation

The baseline values of MAP and the resting hemodynamic parameters were not significantly different between the groups. In the sham-operated group 4, there were no

significant hemodynamic changes as compared with the baseline values throughout the experimental period. In this group, tissue samples taken from the intestine exhibited a median grade of injury of 0 and displayed the structure of the normal mucosa.

Significant differences in the Figures and in the Tables are shown by the following symbols: * $p<0.05$ within the groups, X $p<0.05$ Sham-operated vs. SBTX, # $p<0.05$ SBTX vs. SBTX+ETR-p1/fl i.v. or i.g., respectively.

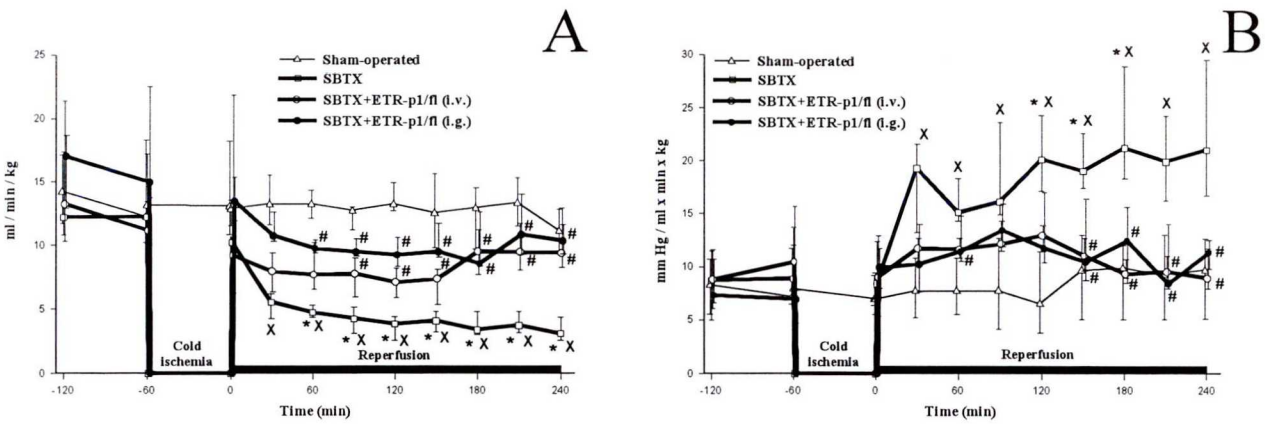
In non-treated group 1, SMVP increased significantly and concomitantly; SMAP decreased significantly during the reperfusion. Similarly, SMAF was significantly decreased after reperfusion as compared with the control group or the baseline values. MVR gradually rose during the 4-h reperfusion phase after SBTX, as shown in Table IV/1 and Figure IV/1.

The treatment with the ET-A receptor antagonist ETR-p1/fl peptide in groups 2 and 3 significantly inhibited the SBTX-induced mesenteric hemodynamic changes (Table IV/1). No significant difference was observed between the two treated groups. In both the systemic i.v. and the i.g. ETR-p1/fl peptide group, the treatment significantly attenuated the fall in SMAF (Figure IV/1/A). 4 h after reperfusion, MVR was not different from the baseline values, in contrast with the progressively rising MVR observed in the non-treated SBTX group (Figure IV/1/B).

Table IV/1 Superior mesenteric arterial and venous pressures

	Time (min)	*Sham-Operated*	*SBTX*	*SBTX+ ETR-p1/fl (i.v.)*	*SBTX+ ETR-p1/fl (i.g.)*
Superior mesenteric artery pressure (mm Hg)	-120	139 (113, 147)	136 (111, 142)	128 (115, 148)	138 (133, 153)
	-60	140 (117, 154)	130 (107, 137)	129 (112, 140)	126 (98.9, 136)
	-59	138 (131, 147)	0 (0, 0)	0 (0, 0)	0 (0, 0)
	-1	136 (127, 153)	0 (0, 0)	0 (0, 0)	0 (0, 0)
	1	140 (117, 148	82.4 (62.9, 109)	102 (68.7, 115)	137 (124, 142)
	60	121 (106, 142)	106 (88.4, 119)	115 (107, 126)	121 (102, 137)
	120	118 (108, 132)	96.9 (74.4, 119)	118 (85.8, 125)	138 (109, 145)
	180	115 (103, 121)	92.7 (79.4, 122)	110 (99.9, 114)	134 (107, 140)
	240	115 (108, 131)	80 (72, 119)*	100 (63.8, 112)*	115 (110, 122)
Superior mesenteric venous pressure (mm Hg)	-120	3.39 (1.4, 4.2)	4.65 (1.88, 5.7)	2.75 (1.29, 4.33)	1.58 (0.72, 3.74)
	-60	3.31 (1.73, 4.24)	5.71 (3.93, 7)	3.6 (2, 6.88)	4.39 (3.18, 5.61)
	-59	3.03 (1.2, 3.5)	0 (0, 0)	0 (0, 0)	0 (0, 0)
	-1	3.44 (1.35, 4.94)	0 (0, 0)	0 (0, 0)	0 (0, 0)
	1	2.8 (1.85, 4.08)	6.19 (4.14, 8.03)	3.96 (1.3, 6.71)	4.75 (3.8, 5)
	60	2.34 (1.3, 3.7)	9.88 (8.68, 11)*,X	3.19 (1.86, 3.66)#	4.87 (4.52, 6.31)#
	120	4.28 (2.63, 5.51)	9.12 (6.65, 10)*	2.09 (0.84, 3.41)#	5.55 (4.98, 6.48)
	180	3.66 (2.39, 4)	7.05 (5.63, 8.87)X	3.86 (3.47, 4.41)#	3.47 (2.52, 4)#
	240	3.25 (2.4, 3.52)	6.66 (6, 7.3)	3.3 (1.67, 4.43)	3 (2, 3.41)#

Figure IV/1 Mesenteric blood flow (A) and mesenteric vascular resistance (B)



The free radical production of the activated granulocytes did not change in the mesenteric blood samples of the sham-operated group throughout the experiments. In the SBTX group, reperfusion was associated with a 2-fold increase in free radical-producing capacity of the leukocytes separated from the effluent venous blood of the intestinal graft. This change was significantly attenuated in group 2 after the systemic administration of the ET-A receptor antagonist. I.g. administration did not influence the elevated free radical-producing capacity of the leukocytes passing the intestinal graft; the value was similar to that observed in the non-treated SBTX group (Table IV/2). No significant difference in peripheral leukocyte count was observed between the SBTX and treatment groups (data not shown).

Table IV/2 Free radical production of granulocytes

	Time (min)	***Sham-operated***	***SBTX***	***SBTX+ ETR-p1/fl (i.v.)***	***SBTX+ ETR-p1/fl (i.g.)***
Free radical production of activated granulocytes (nmol O_2^-/1.5x10^6 cells /min)	-60	3.54 (2.56, 4.95)	4.09 (3.44, 4.75)	5.17 (3.93, 6.02)	4.78 (4.62, 5.11)
	30	4.03 (3.2, 4.94)	5.2 (4, 6.67)	4.53 (3.54, 5.69)	4.55 (3.46, 5.72)
	90	3.88 (2.46, 4.05)	6.78 (5.7, 6.86)	4.36 (3.02, 5.52)	7.66 (4.38, 11.3)
	150	4.26 (3.48, 4.84)	8.04 (6.72, 9)*,X	5.06 (3.95, 5.79)#	9.55 (9.41, 10.7)*
	210	5 (2.88, 6.27)	6.87 (6.82, 8)*,X	4.93 (3.84, 5.24)#	8.17 (7.13, 9.85)*

The mucosal MPO activity was unchanged in the sham-operated group up to the end of the experiments. However, SBTX was associated with a significant elevation in MPO activity 4 h after re-establishment of the blood flow. The reperfusion-induced rise in MPO activity was

significantly decreased after the i.g. and the systemic ETR-p1/fl peptide treatments, and the MPO values were not different from that for the sham-operated animals (Figure IV/2).

Figure IV/3 summarizes the histological scores relating to the mucosal damage of the transplanted intestinal mucosa. In the sham-operated group, relatively normal ileal mucosal morphology was observed in biopsies taken at the end of the experiments. SBTX caused severe mucosal lesions after 4 h of reperfusion. The lesions were often patchy, and the mucosal injury scores frequently ranged between grades 1 and 5 within one field. A marked sloughing of intestinal villi, destruction of the epithelial layer, massive epithelial lifting down the sides of the villi, and complete denudation of the villi were regularly observed. Ulceration, hemorrhage and intraluminal debris formation were also characteristic. Dense infiltration of inflammatory cells into the lamina propria was regularly noted.

Both routes of ETR-p1/fl peptide treatment afforded significant protection against reperfusion-induced mucosal injury. The epithelium appeared relatively well preserved in a majority of the animals. In these sections, the mucosa was often nearly intact, or patchy, mild structural injury and the signs of rapid epithelial regeneration at the tips were observed (Figure IV/4). The semiquantitative Chiu-grading evaluation indicated a significantly lower degree of injury as compared with the non-treated SBTX animals (Figure IV/3). The histologic scoring did not differ between the two treated groups.

Figure IV/2 Mucosal MPO activity

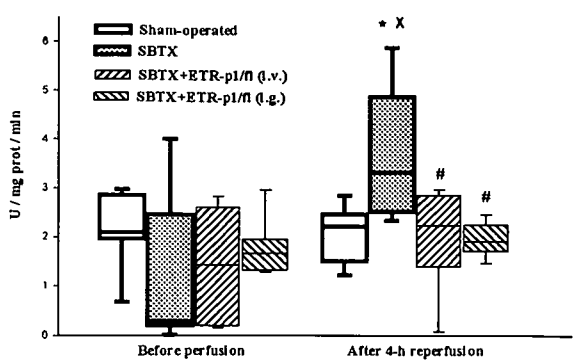


Figure IV/3 Mucosal injury

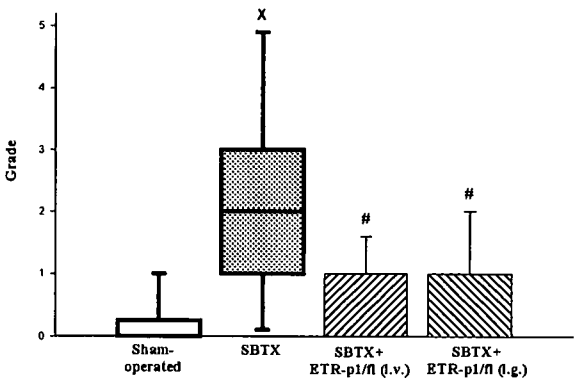
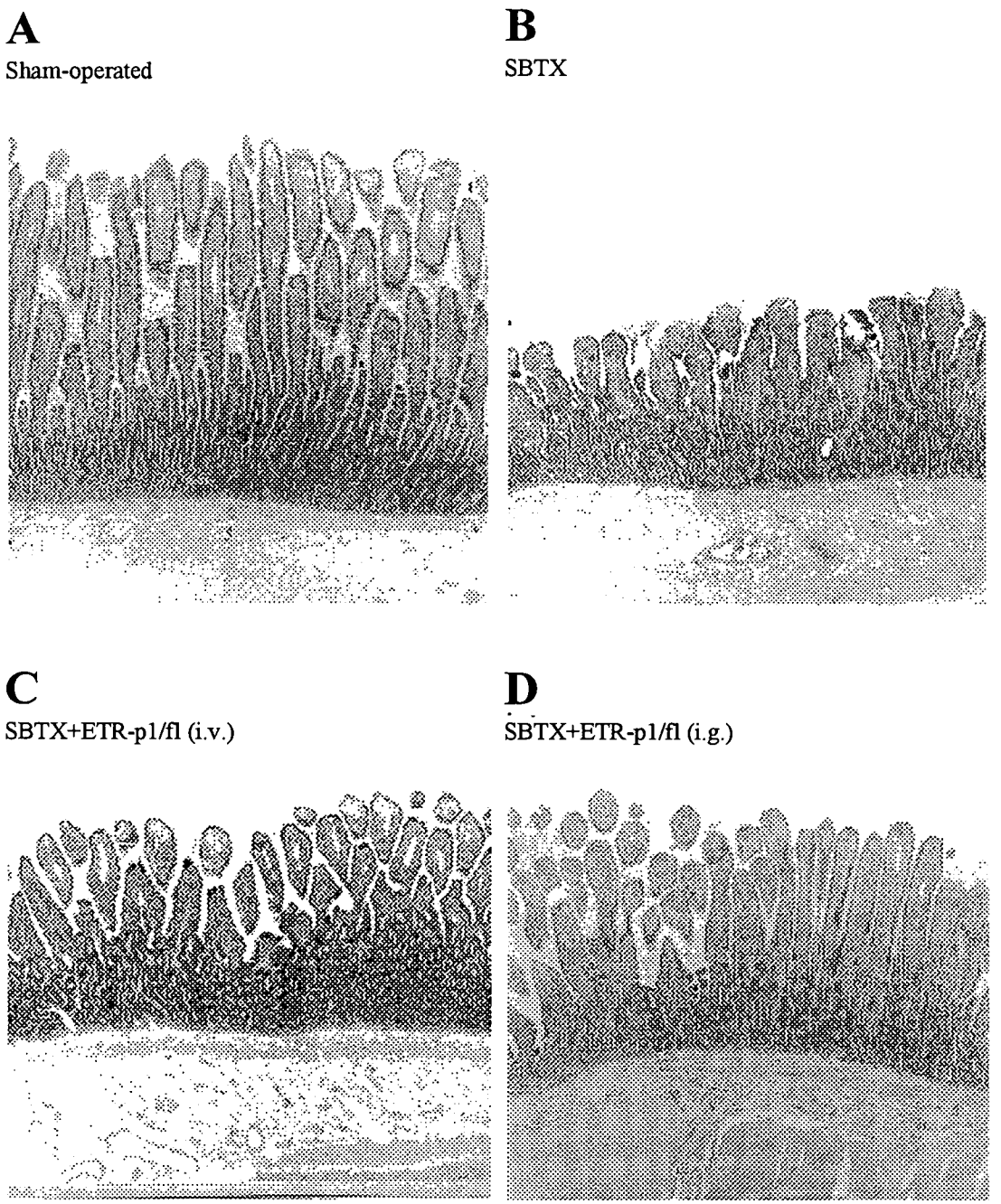


Figure IV/4 Morphological changes in the intestinal mucosa



4. DISCUSSION

4.1. Nitric oxide synthase inhibition in endotoxemia

Sepsis is a typical systemic circulatory disorder, as the initiating stimulus affects the whole circulation. The generalized inflammatory process most frequently induced by ETX results in typical hemodynamic and metabolic changes. Both vasodilation and vasoconstriction can appear, suggesting the presence and importance of vasoactive mediators (Vincent 1998).

Since the hyperdynamic circulatory reaction is characteristic of the early phase of human sepsis (Marik 1998), our large animal model of hyperdynamic endotoxemia can be considered clinically relevant (Deitch 1998). The instrumentation allowed us to monitor the main peripheral (MAP, CO and TPR) and central (myocardial contractility) hemodynamic variables. The actual contractile state of the myocardium was estimated by measuring the end-systolic pressure-diameter relationship, which is a good marker for *in vivo* myocardial contractility (Teitel 1991). The small doses of ETX induced a short hyperdynamic period and significant myocardial depression. It is important to note that after the hyperdynamic phase the cardiovascular parameters in our experiments stabilized, and no signs of endotoxic shock developed during the 8-h observation period.

NO is known to exert vasodilatory and cardiodepressant effects, and it has therefore been suggested that it can play an important role in the mediation of endotoxemic cardiovascular failure (Johnson 1998, Sun 1998). Several studies indicate that only NO overproduction by the enzyme iNOS is responsible for the harmful cardiovascular effects during endotoxemia. iNOS is activated in several cell types during endotoxemia, including cardiomyocytes (Schulz 1992). Although the heart expresses relatively small amounts of iNOS (Liu 1993, Salter 1991), we investigated the activation of eNOS and iNOS in myocardial biopsies, since (because of the short half-life of the molecule) the NO-related cardiac effects may depend on cardiac NO synthesis. It has been reported that iNOS is activated relatively late during endotoxemia (Bateson 1996), and we therefore established an 8-h endotoxemic model, to leave sufficient time for iNOS activation. Additionally, we terminated some of the experiments after 4 h of endotoxemia, to follow the time course of NOS activation. Our

findings are consistent with those of the earlier studies, since the activity of the iNOS isotype increased only after 8 h of endotoxemia. These data also indicate that iNOS-derived NO was not involved in mediating the early hemodynamic changes in our experiments. On the other hand, the eNOS activity was already increased after 4 h of endotoxemia, and was still elevated at the 8th hour of the experiments, suggesting that NO production by eNOS could indeed have a role in the early myocardial depression.

It has been suggested that the inhibition of NO overproduction by using NOS inhibitors can be beneficial in sepsis. The arginine-analog substrates are non-selective inhibitors of both eNOS and iNOS. In our experiments, we applied the non-selective L-NAME. The compound decreased the activities of both NOS isoenzymes after 8 h of endotoxemia, and prevented hypotension and myocardial depression, but decreased CO, and resulted in a marked increase of peripheral resistance. These findings are in agreement with those of earlier studies, involving the use of arginine-analog compounds (Herbertson 1996, Kaszaki 1996). The decreased CO, the reduced blood flow to certain organs, the decreased renal function and the increased microthrombus formation in the microvessels (Henderson 1994, Jourdain 1997, Minnard 1994, Spain 1994) are probably related to the decreased activity of eNOS and suggest the importance of eNOS-derived NO production in endotoxemic conditions.

The aim of selective iNOS inhibition is the attenuation of NO overproduction, without disturbing the normal vasoregulatory function of eNOS. The S-substituted isothiourea derivatives have been described as selective inhibitors of iNOS *in vitro* (Southan 1995). The isothiourea derivative SMT was found to protect against hypotension and to increase survival in a rodent model of sepsis (Southan 1995). The compound improved the peripheral hemodynamics in a large animal endotoxemic model (Liu 1996), and enhanced cardiac performance in myocardial infarction (Wildhirt 1996). We demonstrate for the first time the selective iNOS-inhibitory property of SMT in endotoxemia. It decreased the activity of iNOS without inhibiting eNOS after 8 h of endotoxemia. SMT was also able to protect against the ETX-induced early cardiac depression, but induced a moderate decrease in CO, with increases in TPR and MAP. These hemodynamic effects of SMT cannot be related to iNOS inhibition, since our NOS activity measurements demonstrated the lack of iNOS activation after 4 h of endotoxemia. Although SMT seemed to be selective toward iNOS, according to the 8-h NOS

activity measurements, its early hemodynamic effects suggest a slight eNOS-inhibitory property for the compound when iNOS is not yet activated. This hypothesis was confirmed by earlier observations, demonstrating a significant pressor response after SMT administration in a dose of 0.1 mg/kg, to intact, non-endotoxemic rats, in which the activation of iNOS is not present (Southan 1995).

In Study II, we determined the effects of an another isothiourea derivative, S-(2-aminoethyl) isothiourea, in the same endotoxemic model. It has been described that this compound selectively inhibits iNOS activity *in vitro* and protects against ETX-induced hypotension in rats (Southan 1995). It has also been reported that S-(2-aminoethyl) isothiourea spontaneously rearranges to MEG in the circulation at neutral pH (Southan 1996). This acronym has therefore been used for the compound in our study. MEG exerted favorable effects on the investigated hemodynamic parameters. Although it did not affect MAP, the treatment stabilized CO on a higher level and TPR on a lower level than observed in the endotoxemic control group. The increased CO after MEG treatment may be of importance, because of the increased oxygen demand of metabolically active organs (Ruokonen 1993). The mechanism of this effect can be either the influence on the peripheral vasoconstriction, evolving after the hyperdynamic phase, or the increased myocardial contractility. MEG is an inhibitor of cyclooxygenase, and it is therefore possible that it decreases the formation of vasoconstrictor cyclooxygenase products (Zingarelli 1997). However, the lack of effect of MEG on MAP suggests that the prevention of vasoconstriction can play only a minor role. The reduced TPR after the treatment is rather a result of the increased CO. In a previous study, it has been suggested that the elevated CO after MEG infusion in hemorrhagic shock might be a result of an enhanced cardiac performance (Szabó 1999). In Study II, we measured the myocardial contractility directly, and found a significant protection by the compound against the ETX-induced myocardial depression.

The observed early eNOS activation and the late activation of the iNOS in our endotoxemic model suggest that the early cardiac depression may be based on eNOS-derived NO overproduction, rather than iNOS activation. MEG selectively inhibited iNOS activity and did not affect eNOS after 4, or 8 h of endotoxemia. Thus, the beneficial effect of the compound on myocardial contractility is not attributed to its NOS-inhibitory property.

Besides NO, other mediators and free radicals, which can influence the myocardial contractility are liberated in endotoxemia. Additionally, inflammatory cell deposition within the myocardium can induce a contractility depression, probably by causing tissue injury. Experimental and clinical observations have demonstrated that the inhibition of free radical production and free radical scavengers exert beneficial effects on the hemodynamics in sepsis (Bloomfield 1997, Galley 1997). MEG has been described as a powerful scavenger of the peroxynitrite anion, a free radical formed from NO and superoxide (Szabó 1997). Therefore, we tested the effects of MEG on granulocyte-derived free radical production (since these cells are among the most important sources of free radicals during inflammatory conditions) and on the myocardial accumulation of granulocytes. Our measurements demonstrated a significant rise in both free radical production and tissue MPO activity in response to ETX. MEG attenuated the activation and the tissue accumulation of granulocytes, suggesting that the beneficial effect on the myocardial contractility in this setting may depend on reduced free radical and granulocyte-derived tissue dysfunctions.

The ETX-induced myocardial failure may be influenced by removing the circulating inflammatory products of activated granulocytes. *In vitro* experiments were also performed in which previously unstimulated granulocytes from intact animals were used to clarify the effects of MEG on granulocyte-derived free radical production without the confounding effects of other mediators which could influence the granulocyte functions. We demonstrated that MEG is able to modulate the free radical production of formerly resting granulocytes, and thus the direct free radical-scavenging effect of MEG can not be excluded in the *in vivo* setting.

Our new findings in Studies I and II are that the early myocardial depression in endotoxemia is probably mediated by an increased eNOS activity, an enhanced free radical production and the myocardial deposition of granulocytes, while the contribution of iNOS to the hemodynamic changes in this setting seems to be negligible. Non-selective NOS inhibition by L-NAME results in both beneficial and harmful effects. The NOS activity measurements indicated that SMT and MEG were selective inhibitors of iNOS *in vivo*. We suggest that SMT acted via moderate eNOS inhibition in the early phase of endotoxemia, when iNOS was not yet activated. The protection against early myocardial depression by MEG was mediated by the decrease of granulocyte and free radical-mediated changes. In conclusion, selective iNOS

inhibition can be advantageous in the later phase of endotoxemia, when iNOS-derived NO overproduction would become significant, whereas in the early phase the treatment rather targets granulocyte activation.

4.2. Endothelin-A receptor antagonism in endotoxemia

Previous observations of elevated plasma levels of the ETs during sepsis suggest the role of the ETs in this process (Battistini 1996, Weitzberg 1991). Additionally, it has been established that the ETs are involved in shaping the hemodynamic pattern of hypodynamic sepsis (Szalay 1998). However, the possible relationship between the ETs and the changes in myocardial contractility and the peripheral hemodynamics in hyperdynamic endotoxemia has not been investigated before. Elevated plasma levels of ETs in our study suggest that the ETs could have a role in mediating the cardiovascular changes. The ET-A receptor antagonist ETR-p1/fl peptide significantly prolonged the hyperdynamic circulatory reaction in our model, the CO remained elevated and TPR decreased until the end of the 4-h observation period. Similarly, ETR-p1/fl peptide effectively antagonized the ETX-induced myocardial depression. The first explanation of the effects of this compound on CO and TPR is the prevention of ET-induced vasoconstriction, which might evolve after the hyperdynamic phase in the endotoxemic control animals, as indicated by the increase in TPR. On the other hand, the preserved myocardial function could also contribute to the increased CO after the treatment. The mechanism of this effect is less clear, since the ETs are basically described as positive inotropic substances. However, it has been reported that the positive inotropic effect of the ETs is mediated via ET-B receptor activation (Beyer 1995). Therefore, because of the suggested cross-talk between the ET receptors, the selective antagonism of ET-A receptors with ETR-p1/fl peptide could enhance the effects via the ET-B receptors (including positive inotropy) (Ozaki 1997). On the other hand, we have demonstrated that enhanced free radical production and the increased tissue accumulation of activated granulocytes play a role in the development of early cardiac failure. In Study IV, using the animal model of intestinal transplantation, we found that ETR-p1/fl treatment decreases free radical production and the mucosal accumulation of granulocytes. Thus, we hypothesize that the compound influenced the

activation and myocardial deposition of the inflammatory cells in the endotoxemic model as well.

The new findings in Study III are that ET-A receptor blockade has beneficial effects on myocardial performance and on the compensatory hyperdynamic circulatory reaction in early endotoxemia. Elevated levels of circulating ETs in our hyperdynamic endotoxemic model suggest their role in the mediation of the hemodynamic changes.

4.3. Endothelin-A receptor antagonism in small bowel autotransplantation

Mesenteric ischemia-reperfusion is a classical example of a peripheral circulatory disorder. The initial event affects only a part of the whole circulatory area (the mesenteric vascular bed), but later (especially when bacterial translocation progresses through the injured mucosal barrier) a septic state can also develop. Although, the ischemic conditions and reperfusion are well controlled in transplantation procedures, the reperfusion injury of the mucosa can contribute to the high complication rate of intestinal transplantation.

Mesenteric ischemia-reperfusion is characterized by marked vasoconstriction, free radical-mediated tissue injury and granulocyte deposition within the mucosa (Boros 1995, Eppihimer 1997, Gonzalez 1994). The ETs are potent vasoconstrictors (Rubanyi 1994) and are able to induce leukocyte-endothelial interactions in rat mesenteric venules (Boros 1998), so that their contribution to the pathologic changes during ischemia-reperfusion is likely. To investigate this question further, in Study IV we established a model orthotopic SBTX and tested the effects of the ET-A antagonist ETR-p1/fl peptide in this setting. Autotransplantation was performed to avoid any immunologic reaction; thus, we could investigate the ischemia-reperfusion-related pathologic events separately after transplantation.

The transplantation and reperfusion of the small intestine resulted in a progressive decrease in SMAF and a rise in MVR. The concomitant rise in SMVP could be a sign of postcapillary mesenteric congestion or venous pooling and might also contribute to the fall in arterial perfusion due to a decreased venous return. The prompt and sustained increases in MVR and SMVP suggest that a gradually evolving vasoconstriction was initiated on reperfusion in the mesenteric arterial and venous system, even when SMAP remained relatively

unchanged. The ETR-p1/fl peptide significantly improved the mesenteric perfusion, by decreasing MVR and SMVP and attenuating the fall in SMAF, suggesting that endogenously produced ETs induce vasoconstriction in the mesenteric circulation via the ET-A receptors during reperfusion. Additionally, capillary no-reflow can also contribute to the rise in MVR. The no-reflow phenomenon is a multicausal event; the main factors are interstitial edema, endothelial swelling and disruption and leukocyte plugging within the microvessels (Menger 1997). Filep et al. have shown that ET-1 induces dose-dependent increases in vascular permeability through the activation of ET-A receptors as a consequence of the disruption of the endothelial barrier (Filep 1992). Similarly, it has been demonstrated that ET-1 might act as a promoter of leukocyte rolling in the intestinal microcirculation, and ET-A receptor inhibition decreases the ET-1-induced endothelial-leukocyte interactions (Boros 1998). This indicates that the attenuation of no-reflow can also contribute to the beneficial effects of ETR-p1/fl.

On the other hand, systemic effects of the treatment should also be considered. It has been reported that intestinal ischemia-reperfusion induces myocardial depression (Schlichting 1995). As we demonstrated in Study III, ETR-p1/fl peptide restores the depressed cardiac performance in endotoxemia. Thus, we cannot exclude a positive inotropic effect of the treatment after SBTX as well, probably resulting in an increased CO and improved SMAF. However, since i.g. administration of the peptide was as effective as systemic infusion, the possible effect of the compound on cardiac performance can play only a minor role in this model.

The reperfusion of the intestine was accompanied by a considerable granulocyte deposition in the mucosa and a rise in the free radical production capacity of the circulating granulocytes. Both routes of ETR-p1/fl treatment reduced the mucosal accumulation of the inflammatory cells, as demonstrated by a decrease in MPO activity in the treated groups. Previously it has been reported that ET-1 induces leukocyte-endothelial interactions (the first step of granulocyte extravasation) via the ET-A receptors (Boros 1998). This observation, together with our results, suggests that endogenously produced ETs enhanced leukocyte-endothelial interactions and consequently tissue granulocyte accumulation in our model as well. The enhanced free radical production by the granulocytes can also play an important role in reperfusion-induced tissue injury. Since ET-1 was found to increase the superoxide production

of pre-stimulated granulocytes (Ishida 1990), we hypothesized that ET receptor blockade could reduce the granulocyte-derived free radical production. I.g. administration of the ET-A receptor inhibitor did not affect this parameter; the antagonist was probably bound to the endothelial cells of the graft and did not reach the systemic circulation. However, in the group receiving systemic treatment, the ET-A receptor antagonist significantly attenuated the activity of the circulating granulocytes, demonstrating the role of the ETs in the activation of these cells during reperfusion. Because of the importance of systemic granulocyte activation, we suggest that a therapeutic optimum could be attained with a combination of local and systemic drug delivery.

Transplantation induces severe structural injury to the mucosa. The observed patchy lesions were similar to those occurring after the administration of exogenous ET-1 (Massberg 1998). The effectiveness of ET-A receptor antagonism in the protection against mucosal damage demonstrates the importance of the ETs in the induction of mucosal lesions. The ETR-p1/fl peptide improved tissue perfusion by decreasing MVR, probably via the reduction of vasoconstriction, and might therefore be able to attenuate the hypoperfusion-related tissue injury. On the other hand, the treatment decreased the intramucosal deposition of granulocytes. Since the extravasated inflammatory cells may contribute significantly to tissue injury, through the liberation of cytolytic enzymes, the decreased leukocyte accumulation could also contribute to the protective effect of ETR-p1/fl peptide. Additionally, in the group receiving the ET-A antagonist i.v., the reduced free radical production by circulating granulocytes might further attenuate the mucosal injury, because free radical production during reperfusion is regarded as an important tissue-damaging factor.

The new finding in Study IV is the protective effect of ET-A receptor antagonism against the transplantation-reperfusion-induced pathologic changes. In conclusion, endogenously produced ETs play an important role in mediating the pathologic changes during intestinal transplantation and reperfusion. ET-A receptor inhibition can be beneficial in states accompanied by intestinal ischemia and reperfusion by enhancing the perfusion, decreasing the granulocyte accumulation and preventing the histological damage.

In conclusion, endothelium-derived vasoactive mediators are involved in the pathophysiologic changes during various circulatory disorders.

In endotoxemic conditions, both the vasodilator NO and the vasoconstrictor ETs contribute to the cardiovascular dysfunction, not only by inducing vasodilation or vasoconstriction, but also by influencing the myocardial contractility and inflammatory cell activation. The blockade of ET-A receptors or the inhibition of NO formation can exert favorable effects, but the physiologic vasoregulatory function of eNOS-derived NO should be preserved.

After intestinal transplantation and reperfusion, significant mesenteric vasoconstriction evolved, which indicates that endothelium-derived vasoconstrictor mediators (e.g. the ETs) play a more important role in the pathomechanism than do vasodilators. Blockade of the effects of the ETs has beneficial consequences, by decreasing vasoconstriction-induced hypoperfusion and granulocyte activation, and probably by restoring the balance between the vasodilator and vasoconstrictor mediators.

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