Anesthesiology 2003; 99:685–92

Thoracic Epidural Anesthesia Attenuates Hemorrhage-induced Impairment of Intestinal Perfusion in Rats

Jörn Adolphs, M.D., Diego K. Schmidt, M.D.,* Shaaban A. Mousa, Ph.D.,* Britta Kamin,† Ines Korsukewitz,† Helmut Habazettl, M.D.,‡ Michael Schäfer, M.D.,§ Martin Welte, M.D.‖*

Background: **During hemorrhagic hypotension, sympathetic vasoconstriction crucially contributes to gut mucosal damage. Sympathetic blockade by thoracic epidural anesthesia has been shown to increase mucosal microvascular perfusion and to improve survival after severe hemorrhage in laboratory animals. This study investigates the effects of thoracic epidural anesthesia on intestinal microvascular perfusion during hemorrhagic hypotension in rats.**

Methods: **In 32 anesthetized Sprague-Dawley rats either lidocaine 2% (thoracic epidural anesthesia) or normal saline (control) was infused *via* thoracic epidural catheters. Hemorrhagic hypotension (mean arterial pressure 30 mmHg for 60 min) was induced by withdrawal of blood, which was subsequently retransfused for resuscitation. Functional capillary density and erythrocyte velocity in the mucosa and muscularis were determined by intravital microscopy. Leukocyte–endothelium interaction was studied in postcapillary venules and sympathetic nerve fibers of the intestinal wall were identified by immunohistochemistry.**

Results: **During hypotension functional capillary density was significantly ($P < 0.001$) lower in the muscularis of the control group (median [25/75 percentile]: −46.5% [−59.6/−20.8%] change from baseline) as compared with animals that received thoracic epidural anesthesia (−6.1% [−13.4/1.1%]). There were no differences in erythrocyte velocity between groups throughout the experiment. Leukocyte rolling increased significantly ($P < 0.001$) after resuscitation in control (12 [6/15] *vs.* baseline 2.5 [1/8]) but not in thoracic epidural anesthesia (4 [2.3/7] *vs.* baseline: 5 [3/15.5]). Sympathetic nerve fibers were identified in the muscularis and submucosa but not in the mucosa.**

Conclusions: **During hemorrhagic hypotension and after resuscitation, thoracic epidural anesthesia has beneficial effects on intestinal microvascular perfusion. Because of blockade of sympathetic nerves, thoracic epidural anesthesia prevents perfusion impairment of the muscularis during hypotension and attenuates leukocyte rolling after resuscitation.**

This article is accompanied by an Editorial View. Please see: Sielenkämper AW, Van Aken H: Thoracic epidural anesthesia: More than just anesthesia/analgesia. ANESTHESIOLOGY 2003; 99:523–5.

* Postdoctoral Fellow, † Medical student, § Professor, Department of Anesthesiology and Intensive Care Medicine, Freie Universität Berlin. ‡ Professor Department of Physiology, Freie Universität Berlin, Department of Anesthesiology, Deutsches Herzzentrum Berlin. ‖ Professor, Department of Anesthesiology and Intensive Care Medicine, Klinikum Darmstadt.

Received from the Department of Anesthesiology and Intensive Care Medicine, Freie Universität Berlin, Berlin, Germany. Submitted for publication August 15, 2002. Accepted for publication March 25, 2003. Financial support was provided by the Department of Anesthesiology and Physiology, Freie Universität Berlin, Berlin, Germany. Portions of this work were presented at the Congress of Physiology, Tübingen, Germany, March 17, 2002, and at the annual meeting of the American Society of Anesthesiologists, Orlando, Florida, October 12–16, 2002.

Address reprint requests to Dr. Adolphs, Department of Anesthesiology and Critical Care Medicine, Freie Universität Berlin, Hindenburgdamm 30, D 12200, Berlin, Germany. Address electronic mail to: joern_adolphs@web.de.

THORACIC epidural anesthesia (TEA) with local anesthetics is a widely used technique for intraoperative and postoperative pain control in major surgery. Hypotension due to decreased vascular resistance and reduced cardiac output is a well-known side effect induced by the blockade of thoracic efferent sympathetic fibers.[1,2] As a consequence of hemodynamic depression, regional blood flow to the splanchnic region may decrease with TEA.[1,3,4] In contrast, beneficial effects of TEA on intestinal motility,[5] blood flow,[6] and mucosal pH[7] have been described in abdominal surgery. In a recent study[8] TEA increased mucosal perfusion in the ileum of rats despite significantly lower mean arterial pressure (MAP) in TEA as compared with control animals. Little is known, however, about the effects of TEA in situations of severe blood loss and hypotension. In a canine model of hemorrhagic shock, survival of animals with TEA was significantly higher compared with controls.[9] To this end, there are no data about microcirculatory effects of TEA during and after severe hypotension.

Focusing on the gut with its well-known susceptibility to ischemia,[10,11] this study assessed the effect of TEA on ileal microcirculation in a constant pressure model of hemorrhagic hypotension in rats. Capillary densities and erythrocyte velocities in both the ileal mucosa and the muscularis were determined using *in vivo* fluorescence microscopy. Furthermore, leukocyte–endothelium interaction was examined in submucosal postcapillary venules. Because TEA induces sympathetic blockade and sympathetic activation is a crucial physiologic response of the intestinal circulation to hypotension, the distribution of sympathetic nerve fibers within the intestinal wall was assessed by immunohistochemistry.

Materials and Methods

Animals

Male Sprague-Dawley rats weighing 250–300 g were used in all experiments. All animals were housed in our animal facility and supplied with a standard diet and tap water *ad libitum* until the experiment. The study was approved by the local animal care committee (Berlin, Germany; permit No. G0021/01).

Epidural Catheterization

Epidural catheters were prepared according to the slightly modified method of Bahar *et al.*[12] Briefly, a 200-mm-long polyethylene catheter (PE 10, Portex, Hythe,

Table 1. Experimental Groups

Group (n = 8)	Layer Investigated	Epidural Infusion
I	Mucosa	Lidocaine 2% (TEA)
II	Mucosa	Normal saline (CTRL)
III	Muscularis	Lidocaine 2% (TEA)
IV	Muscularis	Normal saline (CTRL)

CTRL = control; TEA = thoracic epidural anesthesia.

UK) was armed with a monofilamentous suture (Prolene® 4/0, Ethicon Inc., Somerville, NJ) to reduce catheter dead space to 5 μl. To ensure catheter placement at a thoracic level of T11/12, a hub was created 40 mm from the tip using heated glue (LT110, UHU®, UHU GmbH, Bühl, Germany). A sagittal midline incision along the spinal processes of L2 –S1 was made during general anesthesia. The spinal process of L5 was removed and a hole was drilled in the dorsal lamina of L4. The catheter was introduced to the point where the hub reached the drilling hole. Subsequent suture and tape fixation prevented displacement of the catheter. After each experiment, the correct position of the catheter in the epidural space was confirmed by autopsy. Epidural spread of lidocaine was tested with equivalent doses of methylene blue solution 1% (n = 6). Plasma lidocaine concentrations at the end of the experiment were determined by gas chromatography (n = 5).

Experimental Protocol

Animals were allocated to four groups (table 1). Anesthesia was provided by subcutaneous urethane (1.5 g/kg) and intramuscular ketamine (50 mg/kg). Ketamine was supplemented between the measurements, if necessary. An epidural catheter was inserted as described in the previous section. A tracheostomy was performed and animals were mechanically ventilated with room air using a small-animal ventilator (Harvard Apparatus, Edenbridge, UK). The right and left carotid artery were cannulated using polyethylene catheters (PE 50, Portex) for continuous monitoring of MAP and heart rate (HR) and withdrawal of blood. The right external jugular vein was cannulated for continuous infusion of normal saline (3 ml/h) to ensure normovolemia. Arterial blood was analyzed for blood gases, hematocrit and acid–base status repeatedly (Rapidlab 348, Chiron Diagnostics GmbH, Fernwald, Germany). If necessary, ventilation was adjusted to maintain arterial P_{CO_2} within the normal range (40 ± 5 mmHg). During the hypotensive period metabolic acidemia required hyperventilation to a mean P_{CO_2} of 26.4 ± 2.4 (SD). Body temperature was kept constant at 37°C throughout the whole experiment using a warming pad and a rectal temperature probe linked with a control unit (Harvard Electronics, Edenbridge, UK).

A segment of distal ileum was prepared for intravital microscopy and rats were allowed to stabilize for 30 min. All animals received an intravenous bolus injection of normal saline (1 or 2 ml) to hold initial hematocrit within the range of 35–55%. After intravenous injection of 0.2 ml 5% fluorescein isothiocyanate-labeled dextran (molecular weight 150,000; FD 150S, Sigma Aldrich, Deisenhofen, Germany), baseline recordings from 10 randomly chosen mucosal villi (group I and II) and 10 regions of muscularis (group III and IV) were obtained. Subsequently, an epidural injection of lidocaine 2% (30 μl) was administered in group I and III over a period of 15 min. A continuous epidural infusion of lidocaine 2%, with an infusion rate of 100 μl/h (Infusion pump, Harvard Apparatus, South Natick, MA), was started to sustain the level of sympathetic blockade. Control groups (II and IV) received normal saline in a corresponding regimen. 30 min after the start of the epidural infusion a second set of recordings was obtained. Hemorrhagic hypotension was induced within 15 min by incremental withdrawal of blood into a heparinized plastic syringe to a MAP of 30 mmHg. MAP was maintained at this level for 60 min by further withdrawal or retransfusion of blood (constant pressure model). At the end of the hypotension period, another set of images was recorded to study the impact of hemorrhagic hypotension on ileal perfusion with or without epidural sympathetic blockade. For resuscitation, the entire volume of withdrawn blood was retransfused within 15 min. A final set of images was obtained 60 min after resuscitation. On completion of the experiment, the animals were killed by an intravenous injection of pentobarbital (200 mg/kg).

Immunohistochemistry

Six rats were anesthetized with halothane and transcardially perfused with 100 ml of phosphate-buffered saline (pH 7.4) and 300 ml of cold phosphate-buffered saline containing 4% paraformaldehyde (pH 7.4, fixative solution). The terminal ileum was removed, postfixed at 4°C for 30 min in the fixative solution, and cryoprotected at 4°C overnight in phosphate-buffered saline containing 10% sucrose. The tissues were then embedded in tissue-Tek compound (O.C.T., Miles Inc., Elkhart, IN) and frozen at −20°C. Ten-micrometer-thick sections prepared on a cryostat were mounted onto gelatin-coated slides.

Immunofluorescence staining of the sections was performed as described previously.[13] Unless otherwise stated, all incubations were done at room temperature and phosphate-buffered saline was used for washing (three times for 10 min) after each step. The sections were first incubated for 60 min in block solution (phosphate-buffered saline containing 0.3% Triton X-100, 1% bovine serum albumin, 4% goat serum, and 4% horse serum), then overnight at 4°C with mouse antirat tyrosine hydroxylase antibodies (1:1000, Incstar, Minneapolis, MN). This was followed by 1-h incubation with fluorescein isothiocyanate-conjugated horse antimouse secondary antibody (Vector Laboratories, Burlingame, CA). After final washings, the sections were mounted in Vectashield (Vector Laboratories) and analyzed under a

fluorescence microscope (Zeiss, Jena, Germany) with appropriate filters. To demonstrate specificity of staining, either the primary or the secondary antibodies were omitted. These control experiments did not show tryosine hydroxylase staining. For histologic assessment, ileal tissue sections were stained by hematoxylin and eosin.

Preparation of the Ileum

A loop of the terminal ileum was exteriorized through an abdominal midline incision and positioned on an adjustable platform. This experimental setup ensured permanent visualization of mesenteric vessels and prevention of accidental compression and vascular stasis. Separating the intestinal loop from the abdominal wall avoided the transmission of respiratory movements and improved the quality of *in vivo* imaging. For visualization of the mucosa, the ileal segment was opened along its antimesenteric border over a distance of 3 or 4 cm using electrocautery. Subsequently, it was gently cleaned from bowel content, positioned on the stage with the mucosa showing upwards, and loosely covered with polyethylene foil to prevent tissue damage and to allow optimal spread and fixation of the tissue. The ileal segment and its mesentery were constantly superfused with warmed (37°C) bicarbonate-buffered Ringer's solution equilibrated with 5% carbon dioxide and 95% nitrogen. Observation of the muscularis was performed from the serosal side and did not require incision of the bowel wall.

In Vivo *Microscopy*

Microscopy was performed using a Leitz microscope (Leitz, Wetzlar, Germany) equipped with a 150-W xenon lamp (XBO 150 W/1, Osram, München, Germany) and attached to a Ploemo-Pak illuminator (Leitz) with an I_2 (excitation: 450–490 nm, more than 515-nm emission) filter block for epifluorescence illumination. With a ×10 water-immersion objective, the technique allowed for a magnification of approximately ×600. The microscopic picture was transferred to a monitor (Sony, Cologne, Germany) by a low-light camera (CF8/4 FMC C, KAPPA opto-electronics GmbH, Gleichen, Germany) and videotaped (RTV-950, Blaupunkt, Hildesheim, Germany) for off-line analysis.

Microcirculatory Analysis

An investigator who was blinded to the study design performed microcirculatory off-line analysis using a personal computer equipped with a real-time image-processing board. A specially designed software package (Vision3D, Vmorph, Pries AR, Drüsedow S, Berlin, Germany, 1998) was used to determine capillary density and erythrocyte velocity in the mucosa and the muscularis as well as main villus arteriole diameters. Capillaries were manually redrawn on a transparent sheet from subsequent frozen video images (fig. 1). This allowed us to reproduce the complete microvascular network despite temporary blurring of the picture due to gut motility.

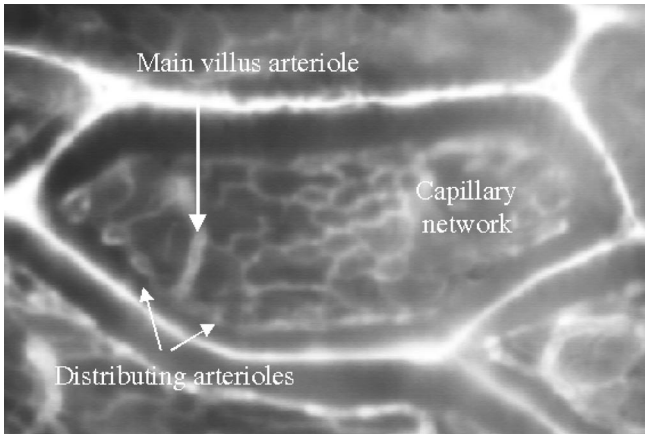

Fig. 1. Frozen video image of a mucosal villus showing important microvascular structures: the terminal feeding arterioles (central villus arteriole with its two distributing arterioles) and capillary network.

Perfused and nonperfused capillaries were determined separately. Using Vision3D, the network was transferred to the computer to obtain capillary lengths (computer-assisted calculation). Observation areas were defined as the boundaries of the villus for the mucosa (fig. 1) and the boundaries of the monitor screen for the muscularis. Functional capillary density (FCD) was defined as the length of erythrocyte-perfused capillaries (cm^{-1}) per observation area.[14] Nonperfused capillary density was defined as the length of capillaries without erythrocyte perfusion over the observation period of 60 s per observation area. The values for FCD and nonperfused capillary density were added to obtain the total capillary density. To obtain one value for capillary density per rat and time point, the capillary lengths of 6–10 regions were added and divided by the sum of the corresponding areas. Erythrocyte velocity was determined using the frame-by-frame method. The distance a fluorescent plasma gap covered over the time of 2–10 frames (40 ms each) was measured and divided by the time. Three values were determined in each observation area and data of 6–10 regions then were averaged. Mucosal erythrocyte velocity was determined in the terminal feeding arterioles (fig. 1). FCD and erythrocyte velocity are reported as percentage change from baseline values. The diameter of the main villus arteriole was assessed at three different segments of the vessel. The three values were averaged to give one value for each individual villus arteriole, and the median value of 10 arterioles was calculated to give one diameter per measurement.

Leukocyte–Endothelium Interaction

To assess leukocyte–endothelium interaction, white blood cells were stained by an intravenous injection of rhodamine 6G (0.1 ml of a 0.2% solution). Using a $N_{2.1}$ filter block (excitation: 515–560 nm, more than 580-nm emission), leukocytes were visualized in submucosal postcapillary venules of 100 ± 14.2 μm diameter. Leukocytes

Table 2. Mean Arterial Pressure, Heart Rate, and Hematocrit

Group	Baseline	30 min after Epidural Infusion	After 60 min of Hypotension	60 min after Resuscitation
MAP (mmHg)				
I (TEA)	115 ± 9	106 ± 16	30 ± 0	51 ± 11*
II (CTRL)	109 ± 13	115 ± 19	30 ± 0	72 ± 23
III (TEA)	123 ± 6	104 ± 16†	30 ± 0	92 ± 22*
IV (CTRL)	118 ± 18	118 ± 13	30 ± 0	121 ± 11
HR (bpm)				
I (TEA)	344 ± 58	347 ± 67	297 ± 55†	301 ± 59†
II (CTRL)	375 ± 53	395 ± 30	368 ± 39	389 ± 47
III (TEA)	389 ± 35	335 ± 56	294 ± 40*	341 ± 69*
IV (CTRL)	388 ± 48	376 ± 19	366 ± 22	436 ± 31
Hct (%)				
I (TEA)	47.4 ± 4.6	41.6 ± 3.0	29.6 ± 4.3	35.5 ± 4.1
II (CTRL)	44.9 ± 4.4	42.4 ± 5.7	26.7 ± 4.1	36.1 ± 7.9
III (TEA)	50.4 ± 3.3	45.6 ± 4.2	31.9 ± 5.0	38.8 ± 3.7
IV (CTRL)	50.4 ± 3.5	47.9 ± 3.9	24.9 ± 6.9†	42.6 ± 2.8

Data are mean ± SD.

* $P < 0.01$, † $P < 0.05$ between groups.

CTRL = control; Hct = hematocrit; HR = heart rate; MAP = mean arterial pressure; TEA = thoracic epidural anesthesia.

moving along the vessel wall at a velocity well below that of other blood cells were defined as rolling leukocytes. Focus was set on the vessel wall at its circumference and leukocytes were counted along a vessel length of 100 μm for 60 s. Three venules were examined in each animal (n = 6 per group) at baseline and 60 min after resuscitation.

Statistical Analysis

All tests were performed using Sigma Stat 2.03 software (SPSS Inc., Erkrath, Germany). Nonparametric data are displayed as medians and quartiles [25th, 75th percentile]. All values in tables are presented as mean ± SD. Blood-loss was compared using paired t test. Two-way repeated measures analysis of variance was applied to all other data. Nonparametric data were ranked before analysis. Student-Newman-Keuls test was used for multiple comparisons. For all statistical tests, significance was assumed at the $P < 0.05$ level.

Results

Epidural spread of lidocaine was tested with analogous doses of methylene blue solution 1% and reached from T6 to L4 (n = 6). Mean ± SD lidocaine plasma concentration in TEA animals at the end of the experiment was 0.54 ± 0.15 μg/ml (n = 5).

Hemodynamic Data

Table 2 shows hemodynamic data, which are comparable at baseline values in all groups. TEA decreased MAP significantly only in group III. During hypotension, HR decreased in TEA animals but not in controls. After resuscitation, MAP and HR were lower in the TEA group than in the respective controls. Hematocrit was significantly lower in control group IV during hypotension. In all groups hematocrit was restored into the normal range (36–54%) after retransfusion of shed blood. During hemorrhage, blood loss was comparable in all groups (group I: 2.0 ± 0.2, group II: 2.2 ± 0.1, group III: 1.9 ± 0.1, group IV: 2.2 ± 0.1 ml/100 g).

Acid–Base Status

Table 3 shows pH and base-excess data in TEA and control groups. Besides an increase in pH after epidural infusion of lidocaine 2%, TEA prevents a severe decrease

Table 3. Acid–Base Status

Group	Baseline	30 min after Epidural Infusion	After 60 min of Hypotension	60 min after Resuscitation
pH				
I+III (TEA)	7.33 ± 0.05	7.37 ± 0.05*	7.34 ± 0.07	7.33 ± 0.07
II+IV (CTRL)	7.33 ± 0.05	7.33 ± 0.06	7.24 ± 0.06†	7.33 ± 0.07
BE (mmol/L)				
I+III (TEA)	−6.0 ± 1.9	−5.2 ± 1.9	−10.2 ± 2.8	−8.1 ± 2.4
II+IV (CTRL)	−5.9 ± 2.1	−6.7 ± 1.6	−15.8 ± 2.5†	−8.6 ± 1.2

Data are mean ± SD.

* $P < 0.05$ *vs.* control group; † $P < 0.01$ *vs.* TEA group.

BE = base excess; CTRL = control; TEA = thoracic epidural anesthesia.

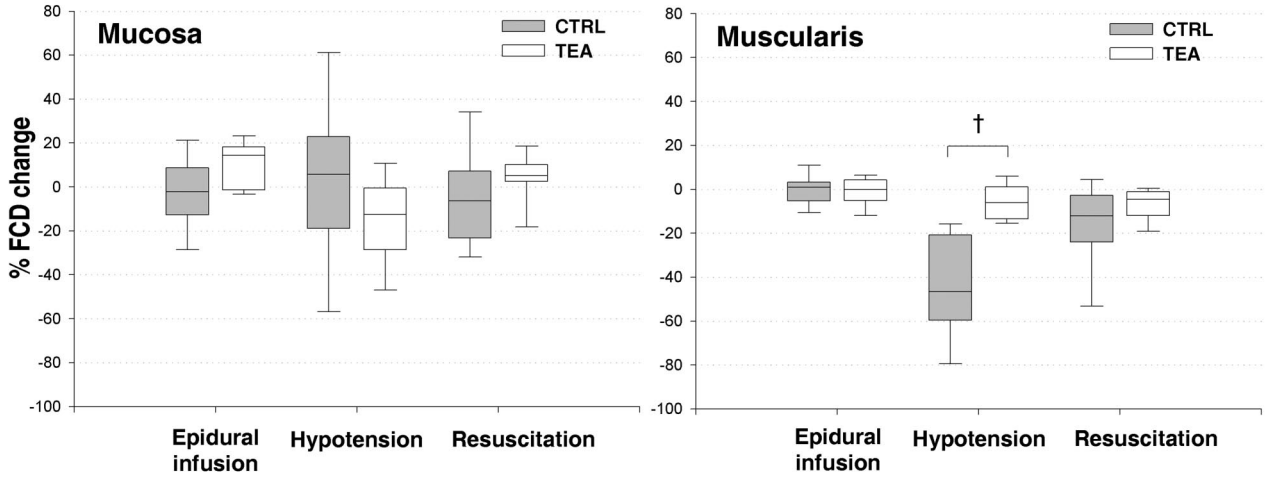

Fig. 2. Change of functional capillary density (FCD) (%) from baseline in the mucosa and the muscularis of thoracic epidural anesthesia (TEA) and control (CTRL) animals. *Box plots* represent median, quartiles, and range. †$P < 0.001$ between groups.

in pH and base excess during hypotension. Mean ± SD pH of the blood after 1 h of storage was 7.19 ± 0.05.

Microvascular Perfusion of the Terminal Ileum

Values for total capillary density were comparable between groups, indicating equivalent microvascular conditions after tissue preparation (group I: 505.4 ± 70.6, group II: 555.1 ± 156.3, group III: 310.3 ± 19.8, group IV: 299.5 ± mm^{-1}). Figure 2 shows the change of functional capillary density from baseline values in the mucosa and the muscularis. In the mucosa, no significant changes were seen during the experiment. In the muscularis, hemorrhagic hypotension significantly impaired capillary perfusion in the control group but not in the TEA group.

Figure 3 shows the change in erythrocyte velocity during the experiment. Epidural infusion of lidocaine 2% or normal saline had no significant effect on erythrocyte velocity. During hypotension velocity is reduced in the muscularis only. After resuscitation erythrocyte velocity is restored. No differences between groups could be seen in either layer throughout the experiment. In both groups, diameters of main villus arterioles did not vary significantly during the experiment (CTRL: 10.4 ± 1.8, 9.3 ± 1.4, 10.6 ± 1.4, 10.1 ± 1.3 μm; TEA: 11.4 ± 1.6, 10.1 ± 2, 9.9 ± 1.3, 10.2 ± 1.5 μm).

Immunohistochemistry

Figure 4 shows hematoxylin-eosin staining (A) and immunohistochemistry (B–D) of the terminal ileum. Perivascular tyrosine hydroxylase-immunoreactive nerve fibers were abundant in the muscularis (B and C) and submucosa (D), but were not detected in the mucosa (D) of the rat terminal ileum.

Leukocyte–Endothelium Interaction

Figure 5 shows that leukocyte rolling increased significantly in the control group after resuscitation but

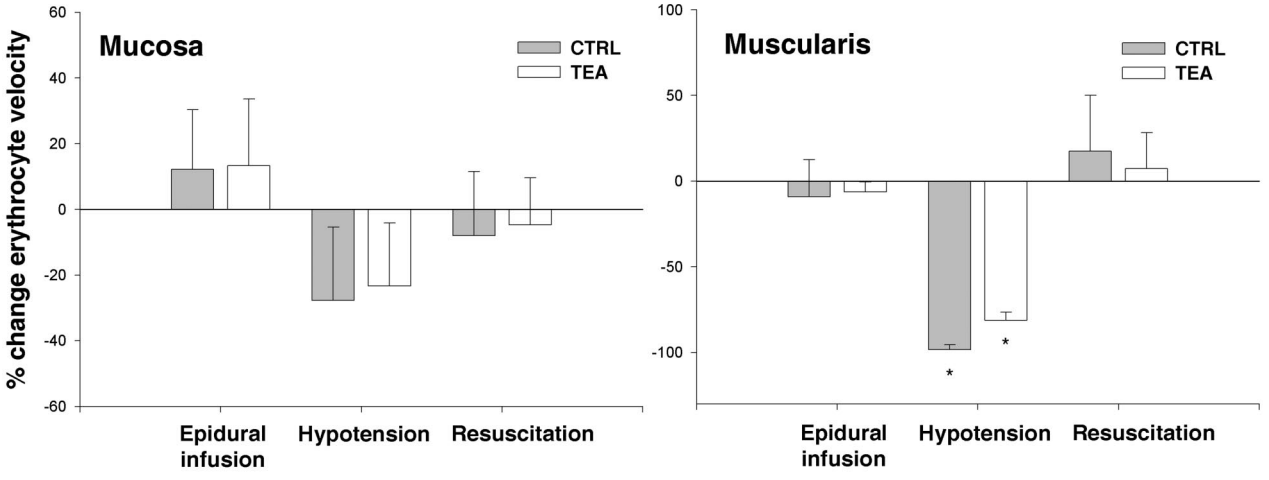

Fig. 3. Change of erythrocyte velocity (%) from baseline in the mucosa (*left*) and the muscularis (*right*) of thoracic epidural anesthesia (TEA) and control (CTRL) animals. Values are given as mean ± SD. *$P < 0.001$ *versus* baseline.

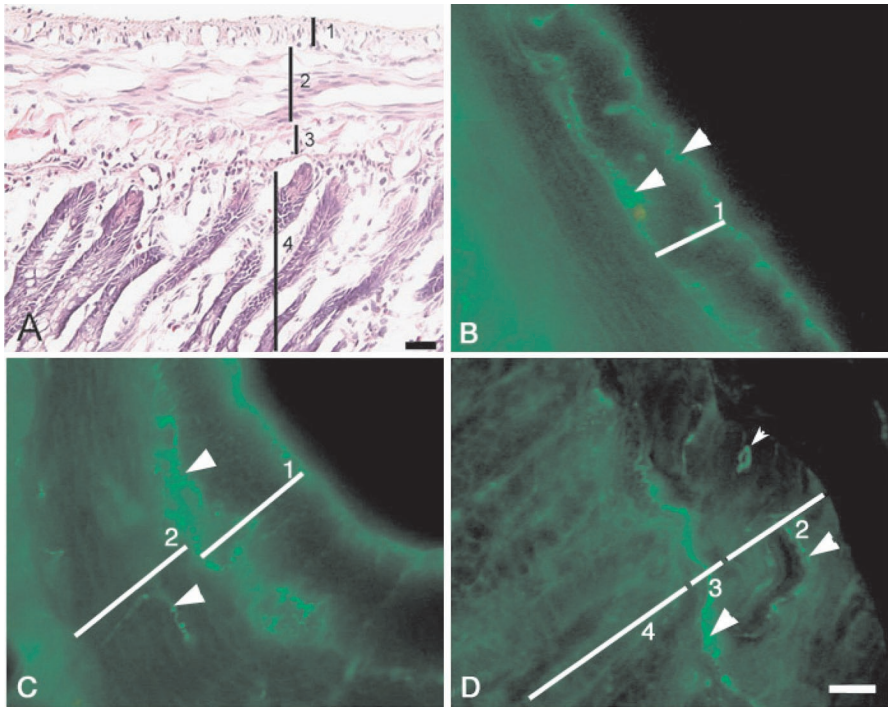

Fig. 4. Distribution of perivascular sympathetic nerve fibers in the ileal wall. Hematoxylin-eosin staining (*A*) shows the different layers: *1* = longitudinal muscularis propria, *2* = circular muscularis propria, *3* = submucosa, *4* = mucosa. Tyrosine hydroxylase-positive immunofluorescence shows sympathetic nerve fibers (*arrowheads*) in the muscularis (*B* and *C*) and submucosa (*D*). No sympathetic nerve fibers were identified in the mucosa of the ileum (*D*). Scale bars = 20 μm.

remained unchanged in the TEA group. Diameters of postcapillary venules did not differ between groups at baseline and after resuscitation (CTRL: 99.8 ± 14.1, 101.8 ± 14.2 μm; TEA: 102.1 ± 10.9, 105.2 ± 17.1 μm).

Discussion

The principal findings of this study are that (1) hemorrhagic hypotension distinctly affects ileal microvascular perfusion, and the capillary perfusion deficit is limited to the muscularis; (2) sympathetic innervation of vessels was detected in all layers of the gut except the mucosa; (3) TEA blocks sympathetic activation and thus inhibits hypotension-induced impairment of capillary perfusion in the muscularis despite a decrease in MAP; (4) TEA prevents systemic acidemia and a severe decrease in base excess during hypotension; and (5) TEA inhibits the increase of rolling leukocytes after hypotension and resuscitation.

To quantitatively assess the effects of hemorrhagic hypotension, TEA, and the combination of both on the transmural microvascular perfusion of the gut, we determined FCD and erythrocyte velocity of both the mucosa and muscularis in rats. Apart from the fact that tissue supply with oxygen also takes place by transmural oxygen loss through precapillary microvessels,[15] FCD and blood flow represent major parameters of tissue oxygenation.[14,15]

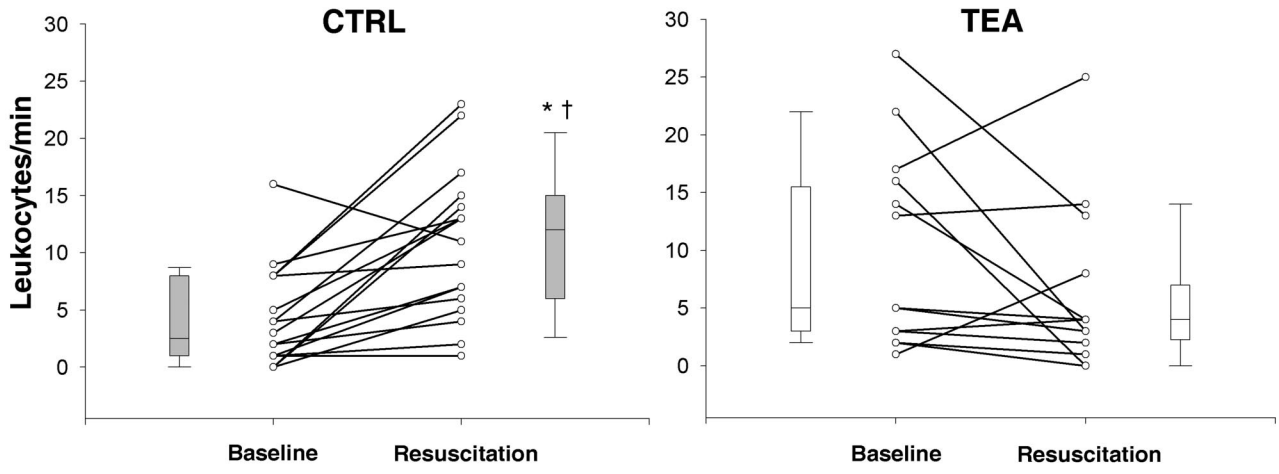

Fig. 5. Number of rolling leukocytes per minute in postcapillary venules. Values at baseline and 60 min after resuscitation in thoracic epidural anesthesia (TEA) and control (CTRL) animals. Values are given as scatter plot with corresponding box plots. **P* < 0.001 *versus* baseline, †*P* < 0.01 *versus* TEA.

Hemodynamic Data

During the constant-pressure hypotension period, HR was significantly lower in TEA animals (table 2). Sixty minutes after resuscitation, MAP and HR were significantly altered between groups, and differences in MAP and HR seen with TEA are most likely due to sympathetic blockade of nerve fibers innervating resistance vessels and the heart, respectively. Because methylene blue staining has revealed epidural spread to a thoracic level of T6, we speculate that the local anesthetic effect exceeds the upper level of the dye, a finding that has been described previously.[16]

Hematocrit in group IV was slightly but significantly lower compared to other groups during hemorrhage. Hemodilution studies in rats have shown that the critical hematocrit for intestinal oxygen supply is approximately 16%,[17] which is far lower than the value reached in group IV (24.9%). Also, hemodilution to a hematocrit of 25% does not affect the ability of the gut to maintain oxygen consumption during hypotension (MAP 42 mmHg).[18] Moreover, a decrease in tissue oxygenation should cause an increase in capillary exchange surface and blood flow than a decrease. This implies that the decrease in FCD (fig. 2, right) is due to an alteration in perfusion pressure rather than an alteration in hematocrit.

Intestinal Microvascular Perfusion during Hypotension and after Resuscitation.

The decrease in erythrocyte velocity observed during hemorrhage in the ileal muscularis (fig. 3) is in agreement with the common finding of splanchnic hypoperfusion as a regulatory response to systemic hypotension.[19,20] Our results further show that during hemorrhagic hypotension capillary perfusion is reduced in the muscularis but not in the mucosa. This transmural redistribution is in line with the results of studies that found a shift of blood flow towards the more vulnerable mucosa when perfusion pressure is decreased.[21–23]

Although multiple factors regulate intestinal blood flow, we hypothesize that the redistribution of intestinal capillary perfusion in favor of the mucosa during hemorrhage is mainly due to sympathetically mediated vasoconstriction. This hypothesis is supported by our immunohistologic findings (fig. 4), which show rich sympathetic innervation in vessels of the submucosa and muscularis but not of the mucosa. Accordingly, the mucosa is far less affected by sympathetic stimulation as compared to the muscularis.[24] During sympathetically mediated vasoconstriction of large submucosal arterioles, mucosal arterioles may even dilate as a result of an autoregulatory escape.[25,26]

Effects of Thoracic Epidural Anesthesia on Intestinal Microvascular Perfusion

Under baseline hemodynamic conditions, TEA did not notably alter mucosal FCD (fig. 2, left). Accordingly, Sielenkämper *et al.*[8] reported that epidural bupivacaine had no significant effect on total intercapillary area size, an analogous value for FCD. They described, however, an increase in erythrocyte velocity and a considerable shift from intermittent to continuous perfusion of mucosal capillaries. Both effects could not be confirmed in our experiments (perfusion data not shown). During subsequent hemorrhagic hypotension, TEA completely prevented the decrease in FCD in the muscularis. Because TEA did not notably alter erythrocyte velocity but led to a significant increase of perfused capillaries during hypotension, an overall increase in blood flow to the intestine must be postulated.

Diameters of the main villus arteriole were not considerably altered by epidural lidocaine. Congruent with our results, neither epidural bupivacaine[8] nor surgical denervation of major mesenteric vessels[27] had an effect on the diameter of the villus arteriole. This finding again is explained by scarce adrenergic innervation of the ileal mucosa shown in our immunohistochemical studies. Possible sites for the neural control of intestinal blood flow are parallel (muscularis) and serially connected upstream arterioles (submucosa, mesenterium). Indeed, vasoconstriction of large supplying mesenteric arterioles was observed during hemorrhagic hypotension.[26,28,29]

Effects of Epidural Lidocaine on Systemic pH and Base Excess

Severe metabolic derangements are often seen with hemorrhagic shock in laboratory animals.[30–32] As shown in table 3, TEA prevents metabolic acidemia and reduces the dramatic decrease in base excess during hypotension. Although these values are restored in controls after resuscitation, they represent important prognostic factors for survival after hemorrhagic shock.[31] A reduction of gastric and intestinal pH by means of TEA has been demonstrated by others.[7,33–35] Whether alterations in systemic acid–base status are based on changes in gastrointestinal pH cannot be stated.

Leukocyte–Endothelium Interaction

Leukocyte–endothelium interaction after ischemia/reperfusion is a well-described phenomenon that contributes to postischemic tissue injury.[36,37] Prevention of leukocyte rolling, the first step of the inflammatory reaction to low-flow states, attenuates reperfusion injury[38] and increases survival after hemorrhagic hypotension in rats.[39] We examined the influence of TEA on posthemorrhage leukocyte–endothelium interaction. We chose submucosal postcapillary venules for assessment of leukocyte rolling because these vessels drain blood from muscularis and mucosa. Figure 5 shows that epidural lidocaine prevented the rise in the number of rolling leukocytes after hemorrhage and resuscitation observed in control animals. Although a systemic effect of lidocaine cannot be completely excluded, a mean plasma concentration of 0.51 μg/ml seems to be too low to influence leukocyte trafficking. Effect on superoxide anion release of neutrophils is re-

ported at plasma levels of 1.0–5.6 μg/ml in humans.[40] In a rabbit model of hydrochloric acid aspiration, lung leukosequestration was reduced at lidocaine plasma concentrations of 1.2–2.5 μg/ml.[41]

Because venule diameters and arteriolar blood cell velocity are not altered by TEA, an augmentation of venular hemodynamic forces as a possible explanation for decreased leukocyte rolling with TEA is unlikely. We therefore postulate that by improving tissue oxygenation in the intestine during hypotension, TEA reduces activation of endothelium and leukocytes.

In conclusion, this study shows that TEA, despite its depressant effect on HR and MAP, improves microvascular perfusion of the small intestine during acute hemorrhagic hypotension in rats. During hemorrhage, TEA preserves capillary perfusion of the muscularis, most likely due to effects on upstream arterioles and arteries. Furthermore, TEA prevents severe systemic acidosis and inhibits intestinal leukocyte rolling, both important factors for survival after hemorrhagic shock. The specific effect of TEA on microvascular perfusion of the muscularis is in accordance with the lack of sympathetic nerve fibers within the mucosa. Further investigations are needed to assess the effects of regional sympathetic blockade on mesenteric arterioles and blood flow distribution to other organs during hemorrhage.

The authors thank Ursula Hilse, M.T.A., Department of Anesthesiology, Deutsches Herzzentrum Berlin, Berlin, Germany, for her excellent technical assistance and outstanding commitment to this work, and Professor Christoph Stein, M.D. (Head, Department of Anesthesiology) and Professor Axel Pries, M.D. (Head, Department of Physiology), both of Freie Universität Berlin, Berlin, Germany for their constant support.

References

1. Greitz T, Andreen M, Irestedt L: Effects of ephedrine on haemodynamics and oxygen consumption in the dog during high epidural block with special reference to the splanchnic region. Acta Anaesthesiol Scand 1984; 28:557–62
2. Otton PE, Wilson EJ: The cardiocirculatory effects of upper thoracic epidural analgesia. Can Anaesth Soc J 1966; 13:541–9
3. Kennedy WF Jr, Everett GB, Cobb LA, Allen GD: Simultaneous systemic and hepatic hemodynamic measurements during high peridural anesthesia in normal man. Anesth Analg 1971; 50:1069–77
4. Sivarajan M, Amory DW, Lindbloom LE: Systemic and regional blood flow during epidural anesthesia without epinephrine in the rhesus monkey. ANESTHESIOLOGY 1976; 45:300–10
5. Ahn H, Bronge A, Johansson K, Ygge H, Lindhagen J: Effect of continuous postoperative epidural analgesia on intestinal motility. Br J Surg 1988; 75:1176–8
6. Johansson K, Ahn H, Lindhagen J, Tryselius U: Effect of epidural anaesthesia on intestinal blood flow. Br J Surg 1988; 75:73–6
7. Kapral S, Gollmann G, Bachmann D, Prohaska B, Likar R, Jandrasits O, Weinstabl C, Lehofer F: The effects of thoracic epidural anesthesia on intraoperative visceral perfusion and metabolism. Anesth Analg 1999; 88:402–6
8. Sielenkämper AW, Eicker K, Van Aken H: Thoracic epidural anesthesia increases mucosal perfusion in ileum of rats. ANESTHESIOLOGY 2000; 93:844–51
9. Shibata K, Yamamoto Y, Murakami S: Effects of epidural anesthesia on cardiovascular response and survival in experimental hemorrhagic shock in dogs. ANESTHESIOLOGY 1989; 71:953–9
10. Zabel DD, Hopf HW, Hunt TK: Transmural gut oxygen gradients in shocked rats resuscitated with heparan. Arch Surg 1995; 130:59–63
11. Baker JW, Deitch EA, Li M, Berg RD, Specian RD: Hemorrhagic shock induces bacterial translocation from the gut. J Trauma 1988; 28:896–906
12. Bahar M, Rosen M, Vickers MD: Chronic cannulation of the intradural or extradural space in the rat. Br J Anaesth 1984; 56:405–10
13. Mousa SA, Machelska H, Schafer M, Stein C: Co-expression of beta-endorphin with adhesion molecules in a model of inflammatory pain. J Neuroimmunol 2000; 108:160–70
14. Nolte D, Zeintl H, Steinbauer M, Pickelmann S, Messmer K: Functional capillary density: An indicator of tissue perfusion? Int J Microcirc Clin Exp 1995; 15:244–9
15. Tsai AG, Friesenecker B, Mazzoni MC, Kerger H, Buerk DG, Johnson PC, Intaglietta M: Microvascular and tissue oxygen gradients in the rat mesentery. Proc Natl Acad Sci U S A 1998; 95:6590–5
16. Schulte-Steinberg O, Rahlfs VW: Spread of extradural analgesia following caudal injection in children: A statistical study. Br J Anaesth 1977; 49:1027–34
17. van Bommel J, Siegemund M, Henny CP, Trouwborst A, Ince C: Critical hematocrit in intestinal tissue oxygenation during severe normovolemic hemodilution. ANESTHESIOLOGY 2001; 94:152–60
18. Mesh CL, Gewertz BL: The effect of hemodilution on blood flow regulation in normal and postischemic intestine. J Surg Res 1990; 48:183–9
19. Price HL, Deutsch S, Marshall BE, Stephen GW, Behar MG, Neufeld GR: Hemodynamic and metabolic effects of hemorrhage in man, with particular reference to the splanchnic circulation. Circ Res 1966; 18:469–74
20. Wang P, Hauptman JG, Chaudry IH: Hemorrhage produces depression in microvascular blood flow which persists despite fluid resuscitation. Circ Shock 1990; 32:307–18
21. Lundgren O, Svanvik J: Mucosal hemodynamics in the small intestine of the cat during reduced perfusion pressure. Acta Physiol Scand 1973; 88:551–63
22. Redfors S, Hallback DA, Sjovall H, Jodal M, Lundgren O: Effects of hemorrhage on intramural blood flow distribution, villous tissue osmolality and fluid and electrolyte transport in the cat small intestine. Acta Physiol Scand 1984; 121:211–22
23. Gosche JR, Garrison RN: Prostaglandins mediate the compensatory responses to hemorrhage in the small intestine of the rat. J Surg Res 1991; 50:584–8
24. Shepherd AP, Riedel GL: Intramural distribution of intestinal blood flow during sympathetic stimulation. Am J Physiol 1988; 255:H1091–5
25. Theuer CJ, Wilson MA, Spain DA, Edwards MJ, Garrison RN: Alpha-adrenergic receptor antagonism prevents intestinal vasoconstriction but not hypoperfusion following resuscitated hemorrhage. J Surg Res 1995; 59:292–8
26. Fruchterman TM, Spain DA, Wilson MA, Harris PD, Garrison RN: Selective microvascular endothelial cell dysfunction in the small intestine following resuscitated hemorrhagic shock. Shock 1998; 10:417–22
27. Bohlen HG, Hutchins PM, Rapela CE, Green HD: Microvascular control in intestinal mucosa of normal and hemorrhaged rats. Am J Physiol 1975; 229:1159–64
28. Watkins JM, Spain DA, Krysztopik RJ, Downard PJ, Wilson MA, Garrison RN: Heparan preserves intestinal perfusion after hemorrhage and resuscitation. J Surg Res 1996; 66:154–8
29. Scalia SV, Taheri PA, Force S, Ozmen V, Lui D, Fish J, Hansen D, Chambers R, Flint L, Steinberg S: Mesenteric microcirculatory changes in nonlethal hemorrhagic shock: Khe role of resuscitation with balanced electrolyte or hypertonic saline/dextran. J Trauma 1992; 33:321–5
30. Kerger H, Waschke KF, Ackern KV, Tsai AG, Intaglietta M: Systemic and microcirculatory effects of autologous whole blood resuscitation in severe hemorrhagic shock. Am J Physiol 1999; 276:H2035–43
31. Dunham CM, Siegel JH, Weireter L, Fabian M, Goodarzi S, Guadalupi P, Gettings L, Linberg SE, Vary TC: Oxygen debt and metabolic acidemia as quantitative predictors of mortality and the severity of the ischemic insult in hemorrhagic shock. Crit Care Med 1991; 19:231–43
32. Jochem J: Haematological, blood gas and acid-base effects of central histamine- induced reversal of critical haemorrhagic hypotension in rats. J Physiol Pharmacol 2001; 52:447–58
33. Sutcliffe NP, Mostafa SM, Gannon J, Harper SJ: The effect of epidural blockade on gastric intramucosal pH in the peri-operative period. Anaesthesia 1996; 51:37–40
34. Spackman DR, McLeod AD, Prineas SN, Leach RM, Reynolds F: Effect of epidural blockade on indicators of splanchnic perfusion and gut function in critically ill patients with peritonitis: A randomised comparison of epidural bupivacaine with systemic morphine. Intensive Care Med 2000; 1638–45
35. Ai K, Kotake Y, Satoh T, Serita R, Takeda J, Morisaki H: Epidural anesthesia retards intestinal acidosis and reduces portal vein endotoxin concentrations during progressive hypoxia in rabbits. ANESTHESIOLOGY 2001; 94:263–9
36. Engler RL, Dahlgren MD, Peterson MA, Dobbs A, Schmid-Schonbein GW: Accumulation of polymorphonuclear leukocytes during 3-h experimental myocardial ischemia. Am J Physiol 1986; 251:H93–100
37. Grisham MB, Hernandez LA, Granger DN: Xanthine oxidase and neutrophil infiltration in intestinal ischemia. Am J Physiol 1986; 251:G567–74
38. Lefer DJ, Scalia R, Campbell B, Nossuli T, Hayward R, Salamon M, Grayson J, Lefer AM: Peroxynitrite inhibits leukocyte-endothelial cell interactions and protects against ischemia-reperfusion injury in rats. J Clin Invest 1997; 99:684–91
39. Barroso-Aranda J, Zweifach BW, Mathison JC, Schmid-Schonbein GW: Neutrophil activation, tumor necrosis factor, and survival after endotoxic and hemorrhagic shock. J Cardiovasc Pharmacol 1995; 25(suppl 2):S23–9
40. Peck SL, Johnston RB Jr, Horwitz LD: Reduced neutrophil superoxide anion release after prolonged infusions of lidocaine. J Pharmacol Exp Ther 1985; 235:418–22
41. Nishina K, Mikawa K, Takao Y, Shiga M, Maekawa N, Obara H: Intravenous lidocaine attenuates acute lung injury induced by hydrochloric acid aspiration in rabbits. ANESTHESIOLOGY 1998; 88:1300–9