E. Coli Bacteremia-Induced Changes in the Skeletal Muscle Microcirculation Vary with Anesthetics

Andreas S. Lübbe, Patrick D. Harris1, R. Neal Garrison2
Cecilien-Klinik, Bad Lippspringe, Germany; and Departments of Physiology1 and Surgery2, School of Medicine, Health Sciences Center, University of Louisville; Louisville, Ky, USA

Aim. To test if anesthetic procedures change the hemodynamic pattern in animals with experimental septic shock.

Methods. The effect of two anesthetics on systemic hemodynamic and skeletal muscle microcirculatory responses in high cardiac output live E. coli bacteremia was studied in rats and compared to the effect of two other anesthetic procedures in previously published studies.

Results. Baseline blood pressures and cardiac outputs were similar in rats with decerebrate, ketamine/xylazine, pentobarbital or urethane/chloralose anesthesia. There was a relative baseline tachycardia in decerebrate rats. Ketamine/xylazine anesthetized rats had reduced blood pressure, cardiac output, and heart rate. In decerebrate, pentobarbital, and urethane/chloralose anesthesia, cardiac output increased initially during bacteremia but did not remain elevated in pentobarbital anesthesia. Blood pressure and heart rate remained constant in pentobarbital, decerebrate, and urethane/chloralose anesthesia. During bacteremia, cardiac output, blood pressure, and vascular resistance did not change with ketamine/xylazine, but the heart rate increased. Baseline diameters of cremaster muscle large (A1) arterioles were higher in decerebrate anesthesia. A1 arterioles constricted during high cardiac output bacteremia in decerebrate rats, and pentobarbital or urethane/chloralose-anesthetized rats. A4 arterioles in bacteremia dilated in decerebrate and pentobarbital anesthesia, but did not change under urethane/chloralose and ketamine/xylazine anesthesia.

Conclusion. Anesthetics influence baseline systemic variables and the response of systemic hemodynamics of rats to E. coli bacteremia. During bacteremia, anesthetics primarily affect the reactivity of skeletal muscle small arterioles. Ketamine/xylazine anesthesia has the most pronounced effect on systemic and microcirculatory variables and seems to be an inappropriate choice in sepsis experiments in rats.

Key words: anesthesia; arterioles; bacteremia; chloralose; decerebrate state; ketamine; microcirculation; pentobarbital; sepsis; shock; urethane; venules; xylazine

Anesthesia is an integral part of most studies using laboratory animals. The use of anesthetics is necessitated by humane considerations, by legal constraints, and the type of the performed experiment. Traditionally, barbiturates, such as pentobarbital, have been the anesthetics of choice for laboratory rats due to easy administration and ready availability (1). These considerations have often dictated the use of barbiturates rather than a careful matching of an anesthetic agent with its suitability for a particular investigation. However, due to the very different nature of experimental protocols carried out in living animals, no single anesthetic will be optimal in all cases. In addition, anesthetics modulate the function of major organ systems, including the cardiovascular system (2). The introduction of neurologic anesthesia by the decerebration technique, using dissociative anesthetics, such as ketamine, in combination with the tranquilizer xylazine and other injectable anesthetics have provided additional alternatives to barbiturate anesthesia. We (3-6), as well as others (7-11), have shown that anesthetics also have variable effects on the microcirculation. Etomidate, for instance, causes increased dilator capacity in the small arterioles of the skeletal muscle in response to mefenamic acid and ibuprofen, which suggests that this anesthetic triggers the release of dilator prostaglandins in this tissue (3). Halothane attenuates serotonin-induced vasodilation of the small arterioles in the skeletal muscle (5-HT) (4). In shock experiments, Seyde et al (7) showed that cardiac output and regional hemodynamics were least altered in ketamine-anesthetized and less frequently altered in decerebrate versus pentobarbital- and urethane/chloralose-anesthetized rats when the results were compared to those in awake rats. Although these authors used isoflurane anesthesia best simulated the "hemorrhagic shock condition" (7).
Longnecker and Ross (8) found by direct observations of the cremaster muscle microcirculation in halothane-anesthetized rats that large and small arterioles constricted in response to hemorrhage, whereas Hutchins (10) observed less constriction in a similar experiment in the same preparation under urethane/chloralose anesthesia. Wiegman (11) showed similar cremaster muscle microvascular responses to hemorrhage by using pentobarbital and different combined doses of urethane and chloralose.

A major problem in comparing the effect of anesthetics on cardiovascular responses in different studies is data variability, since animals of different size and age are often compared. It was thus the hypothesis of this study that different anesthetic procedures change cremaster muscle microcirculatory responses to high cardiac output in live E. coli bacteremia. We tested this hypothesis by using four different rat groups of the same age, sex, and weight. The animals underwent an identical protocol, the only difference being the type of anesthetic used. We compared the results with those from animals who underwent the decerebration procedure or were anesthetized with pentobarbital, and then received the same treatment as the rats in this study. Experiments in decerebrate animals or those anesthetized by pentobarbital have been published previously (12,13) but the data were interpreted in a completely different context.

Material and Methods
General Animal Preparation
Male Sprague-Dawley rats weighing between 160 and 180 g were used. Rats were fed standard Purina rat chow (Charles River, Indianapolis, In, USA) and water ad libitum until the food was withdrawn twelve hours before the experiment. The operative procedure for systemic hemodynamic measurements has been previously described (12). Briefly, anesthetized rats (see individual groups) were tracheotomized for the airway control, and the left femoral vein was cannulated for infusion of live E. coli bacteria, whereas the left femoral artery was catheterized for blood pressure and heart rate measurements. For determination of cardiac output by transpulmonary thermodilution (12,13), the right common carotid artery was cannulated with a thermistor, the tip of which was placed in the aortic root just above the aortic valve. PE-50 tubing (Braun, Melsungen, Germany) for injection of the saline indicator was positioned in the superior vena cava just cranial to the right atrium via the jugular vein. For the generation of cardiac output curves, a 40 µL saline bolus was injected and changes in arterial blood temperature were recorded as temperature-time curves on a polygraph. Cardiac output was calculated from the area under the curves (12,13).

Microcirculation Preparation
After vessel cannulation, the animals received a 2 mL subcutaneous injection of isotonic saline for fluid resuscitation. The right testicle was then surgically exposed and the cremaster muscle incised by electrocautery anteriorly in an avascular area (14). The (neurovasculary) intact muscle was then suspended over an optical port in a 60 mL tissue bath filled with modified Krebs solution (12). The right testicle was manipulated into the retroperitoneum and the animal and the cremaster bath were positioned on the stage of a binocular microscope (Optiphot, Nikon, Tokyo, Japan) for transillumination. Bath PO2 was maintained at 15-25 mmHg, bath CO2 was held constant at 35-45 mmHg, and bath pH was regulated to be 7.40±0.05 by adjustment of the N2 and CO2 gas flow rate. Cremaster bath and rectal temperatures were maintained stable at 34.0±0.5 and at 36.0±0.5 °C, respectively. Back temperature was monitored to prevent local skin temperature from rising above 40 °C, which could cause movement by spinal reflexes and give the false impression of a lightly anesthetized animal. The microcirculation of the cremaster muscle was directly observed and recorded as previously described (12).

The microvascular anatomy of the cremaster muscle was defined according to the vessel branch order (12,15). The major inflow arteriole was termed first-order arteriole (A1), smaller, second-order arterioles (A2) appeared as almost perpendicular branches from the parent A1 vessels. Successive arteriolar branches from the second-order vessels were designated A3 and A4 vessels. A similar nomenclature applies to the venular system. For this study, diameters of A1, A2, A3 and A4, as well as large V1 and V2 vessels were measured, and in this study we refer to A1 and A2 as large arterioles, to A3 and A4 as small arterioles, and to V1 and V2 as large venules.

Bacteria Preparation
E. coli were cultured from a hospital-acquired pathogenic strain, which was kept frozen in a fresh medium in our laboratory. Prior to each experiment, the bacteria were processed as previously described (12,13) to provide a stationary growth phase culture. After centrifugation and washing, serial dilutions were analyzed spectrophotometrically to provide 6x108 bacteria per 100 g rat body weight. The bacteria were infused iv in 1 mL saline.

Experimental Procedure
There were four groups of animals in this study. Group 1 (N=7) comprised rats anesthetized with the combination of ketamine (45 mg/kg) and xylazine (6 mg/kg) administered ip. They received 33% of these doses when needed (see Discussion) throughout the experimental procedure. These rats received 6x10^8 CFU E. coli/100 g body weight iv over 5 min to give high cardiac output bacteremia. Group 2 (N=8) comprised rats anesthetized with the combination of urethane (800 mg/kg) and chloralose (60 mg/kg) administered ip, and received 10% of these dosages every 45 min throughout the experimental procedure. These rats received 6x10^8 CFU E. coli/100 g body weight iv over 5 min to give high cardiac output bacteremia. Group 3 (N=3) comprised rats anesthetized with ketamine/xylazine as animals from the group 1 and received 1 mL of isotonic saline solution iv over 5 min. Group 4 (N=3) rats were anesthetized with urethane/chloralose as Group 2 animals and received 1 mL of isotonic saline solution iv over 5 min. At the end of each 90 min of the E. coli (groups 1,2) or saline (groups 3,4) protocol, nitroprusside (Sigma, St. Louis, Mo, USA) in 10^-5 mol/L bath concentration was topically applied via the cremaster bath to obtain maximal diameter reading. In all four groups, a 45 min baseline period was allowed for equilibration of the cremaster muscle to the bath environment. After stabilization of the mean arterial pressure, heart rate, and microvascular tone (one indication being brisk vasomotion in the small arterioles), baseline hemodynamic measurements of cardiac output, mean arterial pressure, heart rate, and microvascular diameters were made. Systemic hemodynamic and microcirculatory readings were made in 15 min intervals. Final measurements were taken after the application of nitroprusside. For clarification reasons, data previously published are also included and described in the Results section. Animals from those studies (13) underwent a midscollicular brainstem transection which included an ip injection of urethane (800 mg/kg) and chloralose (60 mg/kg) 5 hours prior to the infusion of 6x10^8 CFU E. coli/100 g body weight in 1 mL of saline over 5 min to give high cardiac output bacteremia (group 5). Other animals in those studies (12) were anesthetized with sodium-pentobarbital (45 mg/kg ip initially and 20% of that dose approximately every 45 min throughout the experimental procedure) and here determined as group 6. The rats also received E. coli bacteria according to the above described protocol. Control experiments from those studies (two extra groups of rats anesthetized via decerebration--group 7 or with pentobarbital and successive saline infusion--group 8) were also included for clarification purposes.

Statistics
The data were expressed as percent changes from baseline values. Group means and standard errors of the mean were calculated for the baseline values and for percent changes over time. Time differences within a group were evaluated (at the p<0.05 level) by one-way analysis of variance (ANOVA). If the ANOVA revealed no time effect within one group, data points at the successive times of measurements were averaged to give an overall pooled data point. These averaged time points were then used for group comparisons (two sample, unpaired t-test; p<0.05 level of significance). If the ANOVA indicated a time effect within one group, data at individual time points of groups were compared between the appropriate groups by the Bonferoni form of multiple range test (p<0.05). Nitroprusside data were analyzed by a one-way ANOVA to test for any changes (at the p<0.05 level) among the groups at the various vessel levels. Differences among the baseline values of the general and hemodynamic variables were tested by unpaired t-test (p<0.05). If there was no time effect in one variable of a group, one-tailed paired t-tests of the time-pooled data were performed to verify the presence or absence of statistically significant (p<0.05) changes from the baseline for that group. This was necessary since the ANOVA on percentage expressed data cannot be used to test for changes from the baseline values which have been normalized to 100% with no variances. If there was a time effect, individual time points were compared to baseline by the Bonferoni procedure (p<0.05).

Results
Systemic Variables
Baseline blood pressures, heart rates, and cardiac outputs were reduced by ketamine/xylazine-anesthesia (Tables 1 and 2).

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Tachycardia was observed in the decerebrate groups and bradycardia and hypotension in the urethane/chloralose groups. Other baseline data among groups were similar (Tables 1 and 2).
Blood pressure was maintained at baseline levels (time zero values) during high cardiac output bacteremia in all experimental groups (Fig. 1). Heart rate increased in the decerebrate and ketamine/xylazine-aneasthetized groups, but not in the pentobarbital and urethane/chloralose groups during high cardiac output bacteremia (Fig. 2). Cardiac output increased in the decerebrate, pentobarbital, and urethane/chloralose groups for at least 60 min after E. coli infusion (Fig. 3). It was maintained longest in the decerebrate and urethane/chloralose groups (Fig. 3). Cardiac output did not change with ketamine/xylazine (Fig. 3). Systemic vascular resistance decreased in the decerebrate, pentobarbital, and urethane/chloralose group initially, but approached baseline levels at 90 min in decerebrate and pentobarbital rats. Resistance did not change under ketamine/xylazine-anesthesia (Fig. 4). There were no changes in systemic hemodynamic variables after saline infusion in any of the control groups over time (Table 3).

Figure 1: Responses of mean arterial blood pressure (BP) to high cardiac output sepsis in decerebrate (D), and pentobarbital (P) or ketamine-xylazine (K) anesthetized rats, 30, 60 and 90 min after E. coli infusion. Anesthetized rats were monitored up to 60 min. Data are means±SEM.

Figure 2: Responses of heart rate (HR) to high cardiac output sepsis in decerebrate (D), pentobarbital (P), ketamine/xylazine (K), and urethane/chloralose (U) anesthetized rats – 30, 60 and 90 min after E. coli infusion. D- and K-anesthetized rats were different (p<0.05).

Figure 3: Responses of cardiac output (Q) to high cardiac output sepsis in decerebrate (D), pentobarbital (P), ketamine/xylazine (K), and urethane/chloralose (U) anesthetized rats, 30, 60 and 90 min after E. coli infusion. Q in D- and U-anesthetized rats increased from baseline at 90 min, Q in P-anesthetized rats at 60 min (p<0.05).

Microcirculatory Variables
There were greater baseline diameters for small arterioles in the decerebrate groups (Tables 1 and 2). Baseline data of large arterioles and venules were within normal range in all groups. First-order arterioles constricted during high cardiac output bacteremia in decerebrate, pentobarbital, and urethane/chloralose-anesthetized rats (Fig. 5). The same arterioles were unresponsive to E. coli-infusion in ketamine/xylazine rats until late in the experiment (Fig. 5). Second-order arterioles did not change from baseline in any group during high cardiac output bacteremia with the exception of a statistically significant constriction of A2 in decerebrate and urethane/chloralose-rats at 60 and 90 min (Fig. 6).

Third-order arterioles dilated during high cardiac output bacteremia under pentobarbital, and in decerebrate- and ketamine/xylazine- or urethane/chloralose-anesthesia (Fig. 7). Fourth-order arterioles dilated significantly in the decerebrate and pentobarbital group (Fig. 8). There was some dilation of A4 arterioles in the ketamine/xylazine and no dilation at 30 and 60 min in the urethane/chloralose-aneasthetized rats during high cardiac output bacteremia.

Figure 4: Responses of systemic vascular resistance (SVR) to high cardiac output sepsis in decerebrate (D), pentobarbital (P), ketamine/xylazine (K), and urethane/chloralose (U) anesthetized rats, 30, 60 and 90 min after E. coli infusion. SVR in D, P, and U anesthetized rats decreased at 30 and 60 min (p<0.05).

Figure 5: Responses of the first-order (A1) arterioles to high cardiac output sepsis in decerebrate (D), pentobarbital (P), ketamine/xylazine (K), and urethane/chloralose (U) anesthetized rats, 30, 60 and 90 min after E. coli infusion. A1 constricted in D, P, and U groups (p<0.05). A1 in K-anesthetized rats did not constrict at 30 min (p>0.05).

Figure 6: Responses of the second-order (A2) arterioles to high cardiac output sepsis in decerebrate (D), pentobarbital (P), ketamine/xylazine (K), and urethane/chloralose (U) anesthetized rats, 30, 60 and 90 min after E. coli infusion. A2 constricted in D- and U-anesthetized rats at 60 and 90 min (p<0.05).

Figure 7: Responses of the third-order (A3) arterioles to high cardiac output sepsis in decerebrate (D), pentobarbital (P), ketamine/xylazine (K), and urethane/chloralose (U) anesthetized rats, 30, 60 and 90 min after E. coli infusion. A3 dilated in D- and P-anesthetized rats at all time points, and in U-rats at 90 min (p<0.05).

Figure 8: Responses of the fourth-order (A4) arterioles to high cardiac output sepsis in decerebrate
(D), pentobarbital (P), ketamine/xylazine (K), and urethane/chloralose (U) anesthetized rats, 30, 60 and 90 min after E. coli infusion. A4 dilated in D-, P-, and K- anesthetized rats at all time points and in U-rats at 90 min (p<0.05). [view this figure]

Figure 9: Responses of the first-order (V1) venules to high cardiac output sepsis in decerebrate (D), pentobarbital (P), ketamine/xylazine (K), and urethane/chloralose (U) anesthetized rats, 30, 60 and 90 min after E. coli infusion. [view this figure]

Figure 10: Responses of the second-order (V2) venules to high cardiac output sepsis in decerebrate (D), pentobarbital (P), ketamine/xylazine (K), and urethane/chloralose (U) anesthetized rats, 30, 60, and 90 min after E. coli infusion. [view this figure]

Diameters of first- and second-order venules did not change from baseline in any group during high cardiac output bacteremia (Figs. 9 and 10). Large and small arterioles and venules did not change in the four control groups after isotonic saline infusion (Table 3).

There was maximal dilation of small arterioles elicited by sodium-nitroprusside in all groups (Table 4).

Table 3: Systemic circulation 30, 60, and 90 min after saline infusion in rats under different anesthesia. All values are given as percentages of the pre-infusion baseline values (mean±SEM) [view this table]

Table 4: Diameters (percent of pre-infusion E. coli or saline baseline; mean±SEM) at time of maximal change after cremaster exposure to 10-5 mmol/L of nitroprusside [view this table]

Discussion
Live E. coli infusion into decerebrate and pentobarbital-anesthetized rats resulted in an increase in cardiac output, stable blood pressure and heart rate, and a decrease in systemic vascular resistance. This systemic hemodynamic situation mimics early clinical (hyperdynamic and hypermetabolic) sepsis (12,13,17,18). In decerebrate and pentobarbital rats, large skeletal muscle arterioles constricted and small arterioles dilated during this high cardiac output bacteremic phase (12,13).

Decerebration
Decerebration provides neurologic anesthesia without the side effects of common anesthetics, if enough time is allowed for the initial anesthetic (used to perform the mid-collicular midbrain transection) to wear off. The decerebration removes all spinal cord and lower cranial nerve (V-XII) afferent input to the thalamus, hypothalamus, and cerebral cortex. Although general hemodynamic and respiratory functions are intact, there is a tachycardia (16,19), apparently due to decrease in vagal tone (16). Other abnormalities include hyperinsulinism several hours after surgery and mild glucose intolerance (20). Although decerebration per se can result in decreased cardiac output (7), our data did not show such decrease in cardiac output after surgery. This reduction in cardiac output after decerebration has been shown to occur when the transection is made behind the hypothalamus (21).

Pentobarbital
Seyde et al (7) showed that skeletal muscle blood flow in hemorrhage (mean values of cremaster, rectus abdominis, gastrocnemius, tibial, and psoas muscles) in awake rats was more similar to that in pentobarbital–anesthetized rats, than to that in decerebrate or urethane/chloralose– anesthetized rats. However, in standard concentration, pentobarbital is believed to depress general hemodynamics and respiration due to its effects on the central nervous system (7,22). Our literature review indicates that initial doses of pentobarbital in many animal experiments are often quite high and doses are often difficult to assess. Yet, the anesthetic supplements primarily determine the depth of anesthesia throughout long experiments. A continuous infusion of 500 µg/kg-1/min-1 pentobarbital in the study performed by Seyde (7) approximates 30% of the initial dose every 30 min. We found an initial dose of 45 mg/kg pentobarbital and supplements of 20% of the initial dose every 45 min to be quite sufficient.

The depth of anesthesia is usually assessed by criteria for the retention of the corneal reflex (retraction of the globe and blinking in response to gently touching the cornea) and criteria for retention of jaw reflexes (head shaking, attempts to masticate and movement of the tongue). Some researchers, however, base their judgment of anesthetic resupplementation primarily on the reaction to a painful stimulus, such as twitching in the tail. We feel that this criterion is not entirely appropriate, since local pain sensation could cause local or spinal cord reflexes which could give a false impression of a lightly anesthetized animal. We believe that the above described criteria or an
acoustic stimulus, such as a short hand clap, is a better and more valid test for the depth of anesthesia. Based on such criteria, our supplement doses of pentobarbital assured good vascular tone in our microcirculatory preparations. Thus, with our pentobarbital doses systemic hemodynamic and skeletal muscle microcirculatory responses to high cardiac output bacteremia were similar to those in the decerebrate group. A depression of pH and pO2 and a decrease in hematocrit, which are sometimes observed under pentobarbital anesthesia (23), were not found in a separate group of pentobarbital rats (unpublished observations) and in dogs before and after nicotine infusion (24).

**Urethane/Chloralose**

Our data show that urethane/chloralose anesthesia maintains cardiac output and systemic vascular resistance. However, with urethane/chloralose there was relative hypotension and bradycardia compared to the decerebrate and pentobarbital groups. Nevertheless, baseline microvascular diameters were within the normal range and nitroprusside maximally dilated small arterioles, which indicates considerable vascular tone during the experiment. In addition, there was brisk vasomotion in the small arterioles before and after E. coli infusion with a frequency of 32 vasomotion cycles per minute and a vasomotion amplitude which was similar to that in the pentobarbital–anesthetized and decerebrate rats. This is contradictory to Faber et al (16) who observed little or no arteriolar vasomotion and less capacity to dilate urethane/chloralose–anesthetized rats.

Large arterioles constricted under urethane/chloralose during bacteremia, as they did in decerebrate and pentobarbital anesthetized rats. On the other hand, small arterioles dilated less with urethane/chloralose during bacteremia than with decerebration or pentobarbital, which suggests that urethane/chloralose alters the responsiveness of the small arterioles of the skeletal muscle to E. coli bacteremia. Although baseline muscle blood flows were similar among the urethane/chloralose, decerebrate, and pentobarbital rats, in response to hemorrhage, muscle perfusion was similar between urethane/chloralose and decerebrate, but different from awake rats (which were closest to pentobarbital animals) (7). Wiegman et al (11) found reduced blood pressures in urethane/chloralose rats compared to pentobarbital animals, but arteriole response of the cremaster muscle to hemorrhage was similar in pentobarbital and urethane/chloralose rats. In our urethane/chloralose rats, baseline heart rate did not increase. In a different study (22), urethane/chloralose also did not affect baseline heart rate, but this variable decreased by 30% in the third (25) and by 12% in the fourth study (26). Hence, these data show that urethane/chloralose can have variable effects in different protocols.

**Ketamine/Xylazine**

There were striking differences in our experimental groups with ketamine/xylazine anesthesia. Muscle rigidity is a common adverse side effect of ketamine anesthesia. Thus, it is frequently combined with tranquillizers, such as xylazine. Ketamine has a rapid onset and provides a great depth of anesthesia (1). In our ketamine/xylazine-anesthetized rats there was pronounced baseline bradycardia, reduced mean arterial pressure and relatively low cardiac output. During sepsis, cardiac output failed to increase and large and small arterioles were poorly reactive to E. coli bacteremia, suggesting a depressed cardiovascular response to E. coli. In contrast, in a different study (9), baseline cardiac output and regional blood flows were changed least with ketamine (compared to enflurane, halothane, and isoflurane) when, on the other hand, ketamine changed the circulatory responses to hemorrhage. However, in this latter study ketamine was used in a different concentration and without xylazine in combination (9). Thus, it appears that xylazine is responsible for some of the macro- and microcirculatory effects that we observed under ketamine/xylazine anesthesia. Our data show a relative tachycardia response in the ketamine-anesthetized rats to E. coli infusion. Although an increase in heart rate can be expected during bacteremia or sepsis (12,17), there was baseline bradycardia in this group before the E. coli infusion. Furthermore, we did not see tachycardia in our other groups, which suggests that the increase in heart rate to “normal” level does not represent a true tachycardia response (when compared to absolute values in other groups) in sepsis.

Ketamine/xylazine also has a variable effect on systemic hemodynamics in different animal species. Baseline heart rate was reduced by ketamine/xylazine in our rat groups, but ketamine/xylazine increased baseline heart rate by 30% in the bat (27). A similar discrepancy was found with pentobarbital. Heart rate in rats did not change under pentobarbital, but it increased by 50% in the bat (5).

In our experiments, ketamine/xylazine prevented early constriction of large and dilation of small arterioles in sepsis, which concurs with the data indicating that ketamine alters microcirculatory responses to stress, such as hemorrhage or bacteremia (5). Since vasomotion frequency (from 32 to 18 cycles per min) and amplitude (qualitative observation) during our sepsis experiments were reduced by ketamine/xylazine, our data support the idea that the combination of ketamine/xylazine...
generally depresses micro-circulatory reactivity to different stress factors in rats. However, vasodilation was inducible with nitroprusside, which suggests that these vessels still possess tone.

General Comments
Baseline diameters of venules were not affected by our anesthetics. These vessels did not change after E. coli infusion regardless of the anesthetic procedure used. This indicates that large venules did not respond to stimuli and mediators that caused systemic hemodynamic and arteriolar changes in E. coli bacteremia.

Anesthetics did not change systemic and skeletal muscle microcirculatory variables from baseline in our saline-infused control groups, suggesting that our preparations and the anesthetic depth were stable over the course of 90 min and that anesthetics themselves did not change microvascular diameters over time.

Taken together, the choice of the anesthetic procedure in experiments with live animals should consider several aspects. Those are researcher’s personal experience, availability of the drug, the animal species, the particular organ system studied, and the time course of the experiment. Among the anesthetic procedures tested in this study, pentobarbital showed similar hemodynamic and skeletal muscle microcirculatory responses to experimental bacteremia as in decerebration. However, there are limitations to the decerebration procedure per se. There is «brain surgery» several hours prior to the experiment. This surgery in a very small organ usually does not cause visible bleeding and edema, but it could result in microscopical pathology. Tachycardia, hyperinsulinemia, and a mild glucose intolerance could influence metabolic alterations during sepsis. Purchase of a stereotactic instrument, etc., impose a money constraint. Five- to six-hour 'waiting period' between initial anesthesia and the beginning of the experiment is a further disadvantage apart from the necessity to continuously balance extracellular fluid volume loss.

Our data further show that skeletal muscle large arteriole constriction in sepsis is attenuated by ketamine/xylazine, whereas urethane/chloralose and ketamine/xylazine diminish skeletal muscle small arteriolar dilation. This agrees with the concept that anesthetics exert their effects primarily in the third- and fourth-order arterioles (2), and with recent data (3,4) which demonstrated an influence of anesthetics on small skeletal muscle arteriole reactivity to serotonin or prostaglandins. The data support the idea that the effects of anesthetics on the microcirculation are unique and not the result of the general state of narcosis.

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Correspondence to:
Andreas S. Lübbe
Cecilien-Klinik
An der Martinusquelle 10
33175 Bad Lippspringe, Germany
tue.luebbe@t-online.de