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Differential effects of selective and non-selective potassium channel inhibitors in ovine endotoxemic shock (macrocirculation) and in a rat model of septic shock (microcirculation)

Running Head: Potassium channel inhibition in vasodilatory shock

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Abstract

BACKGROUND:Potassium-(K^+)-channel inhibitors may increase systemic vascular resistance in vasodilatory shock states.

OBJECTIVE:The purpose of the present study was to compare the macro- and microvascular effects of the adenosine triphosphate-sensitive K^+-channel-(K^+_{ATP})-inhibitor glipizide and the non-selective K^+-channel inhibitor tetraethylammonium (TEA) in ovine endotoxemicshock and septic shock in rats.

DESIGN:Two randomized, controlled laboratory studies.

ANIMALS: Thirty female sheep and forty male Sprague Dawley rats.

SETTING: Animal research facility

INTERVENTION: Systemic hemodynamics were analyzed in ovine endotoxemicshock with guideline-oriented supportive therapy. Sheep were allocated to three treatment groups for 12h: glipizide 10 $mg \cdot kg^{-1} \cdot h^{-1}$, TEA 8 $mg \cdot kg^{-1} \cdot h^{-1}$ or 0.9% saline. The microvascular effects of each drug were evaluated in septic rats (cecal ligation and puncture model) receiving a 2-hour infusion of each study drug: glipizide 20 $mg \cdot kg^{-1} \cdot h^{-1}$; TEA 50 $mg \cdot kg^{-1} \cdot h^{-1}$ or 0.9% saline, respectively, followed by intravital microscopy of villi microcirculation.

RESULTS: Compared to the control group, glipizide infusion increased systemic vascular resistance index and decreased cardiac index and heart rate (HR) in sheep ($p<0.05$), whereas TEA infusion decreased HR and resulted in a decreased survival time ($p=0.001$). In rats, glipizide infusion resulted in an increase in mean arterial pressure and a decrease in HR compared to baseline measurement ($p<0.05$) without relevant effects on the villi

microcirculation.TEA decreased HR and decreased capillary perfusion of the villi microcirculation compared to the sham group (p=0.002).

CONCLUSIONS: Selective inhibition of K^+_{ATP}-channels in ovine endotoxemic shock with glipizide partially restored vasomotor tone without exerting harmful effects on intestinal microcirculationin septic shock in rats.Contrary, non-selective K^+-channel inhibition with TEAshowed deleterious effects in both models, includingimpaired microcirculation and decreased survival time. Future research on glipizide in vasodilatory shock may be warranted.

KEYWORDS: Endotoxemic shock; microcirculation; potassium channel inhibition, septic shock.

Introduction

Sepsis, a life-threatening organ dysfunction due to a dysregulated hostresponse to infection(1), has a persistent high mortality of 20-50% worldwide(2,3). Early death may occur as a result of circulatory failure and inadequate tissue perfusion in association withvasodilatory shock. Catecholamines(in combination with adequate fluid resuscitation) are the first line drugs for the treatment of vasodilatory shock in sepsis(4,5).However, they may be associated with several deleterious side effects especially in high doses(7,6).This "catecholamine resistant" shock andhigh dosages of catecholamines are independently associated with mortality(8,9,6).Therefore, alternative, non-adrenergic vasoconstrictors are necessary.

Data from experimental and clinical studies strongly suggest that K^+-channels play a pivotal role in the pathogenesis of refractory vasodilation in septic shock and endotoxemia(10,12,11).The purpose of the present study was therefore to compare the effects of the adenosine triphosphate-sensitive K^+-channel-(K^+_{ATP})-inhibitor glipizide and the non-selective K^+-channel inhibitor tetraethylammonium (TEA)on macro- and microvascular hemodynamics in endotoxemic and septic shock.It was hypothesized that non-selective K^+-channel inhibition decreases norepinephrine requirements in endotoxemic shock and that selective inhibition of K^+_{ATP}-channelsis superior to a non-selective K^+-channel inhibition in stabilizing systemic hemodynamics without affecting microvascular perfusionin septic shock. To address these questions, two established animal models were used to produce complementary data sets.Systemic and pulmonary hemodynamics were analyzed in an endotoxemic ovine modelwith guideline-oriented hemodynamic therapy(14,13), whereas microvascular effects of both compounds were evaluated in septic rats(16,15).

Materials and Methods

Animals and ethics.

After approval by the Animal Care Committee of the State Government of North-Rhine Westphalia (reference numbers: 9.93.2.10.36.07.126, sheep study; and 9.93.2.10.36.07.201, rat study), 30 healthy adult female ewes were instrumented for continuous monitoring of cardiopulmonary haemodynamics using an established protocol(14,13). In addition, 40 healthy male Sprague Dawley rats were instrumented to perform intravital video microscopy (IVM)intestinal villus microcirculation according to an established protocol(16,15). Figure 1 shows a flow diagram of the experimental protocol.

Anesthesia,instrumentation, and monitoring of sheep.

Sheep were instrumented under balanced anesthesia(detailed information are provided in supplemental digital content, http://links.lww.com/SHK/A693). Afterwards, anesthesia was terminated, and the awake sheep were extubated. To prevent postoperative dehydration, all sheep received an intravenous infusion of 20 $mL \cdot kg^{-1}$of a balanced crystalloid solution (Sterofundin® ISO, B. Braun Melsungen AG, Germany) over one hour following anesthesia. A 24-h period of recovery was allowed.

Experimental protocol (sheep study).

Inclusion criteria for the study were an initial heart rate (HR)<100 $beats \cdot min^{-1}$, core body temperature ≤39.8°C, mean arterial pressure (MAP)≥80 mmHg, mean pulmonary arterial pressure (MPAP)≤20 mmHg and arterial lactate ≤1.2 $mmol \cdot L^{-1}$. All measurements were performed in awake and spontaneously breathing animals housed in metabolic cages.

Following baseline measurements in the healthy state (BL_{sheep}), 2 $mL \cdot kg^{-1} \cdot h^{-1}$ of balanced crystalloids (Sterofundin® ISO, B. Braun Melsungen, Germany) were continuously infused to account for basal fluid requirements. All animals received a continuous infusion of *Salmonella typhosa* endotoxin (Sigma Chemicals, Deisenhofen, Germany, Catalogue # L6386-100mg), which was started at a rate of 5 $ng \cdot kg^{-1} \cdot min^{-1}$ and doubled every hour until MAP fell below 65 mmHg. At this time, endotoxemic shock baseline measurements ($Shock_{sheep}$)were performed and endotoxin infusion was maintained at the respective dose. During induction of endotoxemic shock one animal died and was excluded from the study. If necessary, sheep received a continuous infusion of glucose 40% to prevent hypoglycemia starting at time point $Shock_{sheep}$.

Study therapy was immediately started after$Shock_{sheep}$ measurements.Therefore, the remaining twenty-nine sheep were randomized into three study groups and received a continuous infusion of the individual study drug diluted in 0.9% saline at an infusion rate of 10 $mL \cdot h^{-1}$. The $Glipizide_{sheep}$ group received $10mg \cdot kg^{-1} \cdot h^{-1}$glipizide (glipizide sodium salt; Inotek Pharmaceuticals, Massachusetts, United States; n=10). The dose was based onprevious data in ovine endotoxemia(17,13).The TEA_{sheep} groupwas treated with8 $mg \cdot kg^{-1} \cdot h^{-1}$ TEA (tetraethylammonium chloride; Sigma-Aldrich Chemie GmbH, Munich, Germany; n=9).The TEA dosewas based on own dose-finding experiments to reliably induce vasoconstriction in endotoxemic sheep. The animals of the control group ($Control_{sheep}$; n=10) received 0.9% saline as placebo.

Fluid resuscitation was performed as follows: A bolus of balanced crystalloid (Sterofundin® ISO, 20 $mL \cdot kg^{-1}$), and 6% hydroxyethyl starch solution (HES) 130/0.4 (Voluven® 6%, Fresenius Kabi AG, Bad Homburg, Germany) 10 $mL \cdot kg^{-1}$, were administered after $Shock_{sheep}$ measurement, followed by continuous infusions of crystalloid and HES 6%, at 12 $mL \cdot kg^{-1} \cdot h^{-1}$ and 3 $mL \cdot kg^{-1} \cdot h^{-}$

[1], respectively, throughout the duration of the experiment. If necessary, a bolus of 12 $mL \cdot kg^{-1}$ of crystalloid was administered to maintain a central venous pressure(CVP) of 8-12 mmHg, a pulmonary artery occlusion pressure (PAOP) of 12-15 mmHg and a mixed-venous oxygen saturation(S_vO_2) of ≥65%. Titration of fluid therapy was based on the Surviving Sepsis Campaign guidelines(19,18), which were applicable at the time of study conduction. A MAP of 70±5 mmHg was established by continuous infusion of titrated NE, if necessary.

Hemodynamicvariables were measured continuously and documented hourly. Blood gas analyses were performed hourly.At the end of the 12-h intervention period the ewes were deeply anesthetized with propofol (4 $mg \cdot kg^{-1}$) and killed with a lethal dose of 100 mL potassium chloride solution (7.45%).

Anesthesia and instrumentation of rats.

Forty male Sprague Dawleywere anesthetized and instrumented for cecal ligation and puncture (CLP) according to a standardized protocol (detailed information regarding anesthesia and instrumentation are provided in a supplemental digital content, http://links.lww.com/SHK/A693)(16,15). Thirty rats underwent CLP, whereas ten animals (in the following referred to as $Sham_{rat}$ group) underwent laparotomy without CLP. During the subsequent 22-h period in which peritoneal sepsis developed, animals lived in metabolic cages. Analgesia and fluid therapy were realized by continuous intravenous infusions of fentanyl (4 $\mu g \cdot kg^{-1} \cdot h^{-1}$; Fentanyl Janssen; Janssen-Cilag GmbH) and 0.9% saline (6 $mL \cdot kg^{-1} \cdot h^{-1}$).

Experimental protocol (rat study).

During the 22 hours after the CLP or sham procedure, 8 of 30 rats, who underwent CLP, and 1 of 10 animals of the $Sham_{rat}$ group died. Immediately after this period of 22 hours, baseline measurements were performed (time point $Sepsis_{rat}$)comprising MAP, HR and respiratory rate (RR).Thereafter the remaining 22 septic ratswere randomized into three groups based on their treatment over the following 2 hours:20 $mg \cdot kg^{-1} \cdot h^{-1}$ Glipizide ($Glipizide_{rat}$ group, n = 8); 50 $mg \cdot kg^{-1} \cdot h^{-1}$TEA ($TEA_{rat}$ group, n = 7)or 0.9% saline as placebo ($Control_{rat}$; n = 7). Sham animals also received placebo infusion ($Sham_{rat}$; n = 9).The dose of glipizide was based on a previous study where glipizide reliablyincreased MAP in conscious rats(20), whereas the TEA dose wasbased on data of endotoxemic rats(21).After the 2-hour treatment period measurements of MAP, HR and RR were repeated (time point$Therapy_{rat}$) followed by intravital microscopy of rat villi microcirculation.

Intravital microscopy of rat villi microcirculation.

Immediately after the time point $Therapy_{rat}$, rats were anesthetized by isoflurane insufflation (targeted expiratory fraction 1.0 %; Forene®,**AbbVie Deutschland GmbH & Co. KG,** Wiesbaden, Germany) and continuous intravenous infusion of fentanyl (4 $\mu g \cdot kg^{-1} \cdot h^{-1}$; Fentanyl Janssen; Janssen-Cilag GmbH). Afterwardsthey were prepared for intravital microscopy (IVM) and videos of rat villi microcirculation were recorded in a standardized manner (detailed information are provided in a supplemental digital content, http://links.lww.com/SHK/A693)(22,15). During the entire period of IVM the infusion of study drugs or vehicle, anesthesia and fluid was continued. After the end of the video recordings animals were killed in deep anesthesia with a lethal injection of potassium chloride (7.45%). The analysis of all video recordings was carried out by two independent blinded investigators after the end of the experiment (*off-line;* detailed information areprovided in a supplemental digital

content, http://links.lww.com/SHK/A693). In the videos of rat villi microcirculation, the intercapillary area (ICA) of all capillaries (ICA_{total}), which is inversely correlated to capillary density, was determined (detailed information on the video analysis are provided in a supplemental digital content, http://links.lww.com/SHK/A693). As asurrogate for thefraction ofperfused capillaries, the ICAofcontinuouslyperfusedcapillaries(ICA_{cont}) was determined. The ratio of mean ICA_{cont} to mean ICA_{total} of each animal was calculated and referred to as ratio ICA_{cont}/ ICA_{total}. In addition, the villus terminal arteriolar blood flow ($Q_{terminal}$) was calculated from the diameter of the terminal arteriole ($D_{terminal}$) and the local red blood cell velocity (V_{RBC}).

Aims and study end points.

The sheep study was designed to investigate the systemic effects (long-term) of glipizide and TEA on macrocirculation and survivalin a large animal model of endotoxemic shock with guideline-oriented supportive therapy. Primary study end point of the sheep study was the difference in NE requirements between groups. Secondary endpoints were differences in hemodynamics, oxygen transport variables, organ function and survival times.

The rat study was designed to investigate the immediate (short-term) microcirculatory effects of glipizide and TEA on rat intestinal microcirculation. Primary end point of the rat study was the difference in variables of rat villus microcirculation, whereas differences in MAP, HR and RR were examined as secondary end points.

Statistical analyses of both studies.

Continuous variables were described as median (percentile 25 - percentile 75).Kruskal-Wallis test was used to test hypotheses relative to continuous variables. If applicable, post-hoc

comparisons were conducted using Dunn's test. Comparisons between time points were made using Wilcoxon signed-rank test. Log-rank test was used to analyze survival among groups in the sheep study.

Multivariate models of Generalized Estimating Equations (GEE)(25,23,24) were used for the sheep study data to compare the differences among groups in hemodynamic and metabolic variables throughout the duration of the experiment, assuming a linear evolution in time during the 12 hours after $Shock_{sheep}$(detailed information regarding the use of GEE are provided in a supplemental digital content, http://links.lww.com/SHK/A693).

A 0.05 level of significance was used for all hypothesis tests. Statistical analysis was performed with IBM SPSS® Statistics 24 (IBM, Armonk, United States).

Results

Results of the sheep study

Effects of endotoxin infusion in sheep (n=29).

After median 17 hours [8; 33] endotoxin infusion resulted ina hypodynamic shock with reduced MAP ($p<0.001$ versus BL),increase in HR ($p<0.001$), and decline in CI ($p=0.043$) due to a reduction in stroke volume.In addition,left ventricle stroke work index (LVSWI) ($p<0.001$) and CVP ($p=0.002$) decreased and animals developed fever ($p<0.001$).There were no differences in oxygen delivery or consumption with the onset of shock, whereaslactate increased. Moreover, endotoxin infusion resulted in metabolic acidosis with reduction in base excess (BE, $p<0.001$) and pH ($p=0.001$). MPAP and PVRI increased significantly ($p\leq0.001$). There was a reduction in urinary output ($p=0.003$). Table 1 shows corresponding data to the results mentioned above.

Hemodynamic and metabolic effects of K^+-channel inhibitors.

The initiation of a supportive therapy with fluids and NE led to a hyperdynamic macrocirculation with increases in CI, HR and MAP and continuous decreases in SVRI in all three study groups(detailed information of the course of CI, HR, MAP and SVRI in each study group are provided in a supplemental digital content, http://links.lww.com/SHK/A693). This hyperdynamic macrocirculation was maintained throughout the duration of the experiment. There was no significant difference in MAP among groups during the 12-hour observation period, although animals in the $Glipizide_{sheep}$ group had an overall increased systemic vascular resistance index(SVRI; p=0.001; Table 2) compared to $Control_{sheep}$. Animals in both treatment groups had lower HR compared to $Control_{sheep}$ (vs. $Glipizide_{sheep}$ p=0.013; vs. TEA_{sheep} p=0.017; Table 2).Neither the infusion rate nor the cumulative dose of NE were statistically different among groups, despite tendencies towards lower NE doses in the glipizide versus the control group (Tables 3 and 4).

Compared to $Control_{sheep}$, glipizide infusion was associated with decreased CI (p=0.003; Table 2) andstroke volume index (SVI) (p=0.008; Table 2). Oxygen delivery (p=0.019; Table 3) and consumption (p=0.003; Table 3) were decreased in the $Glipizide_{sheep}$ group compared to $Control_{sheep}$ with no difference in oxygen extraction ratio (O_2ER) or S_vO_2 (Table 3).TEA infusion was associated with increased oxygen consumption (vs. $Glipizide_{sheep}$; p=0.005; Table 3) with no difference in oxygen delivery compared to $Glipizide_{sheep}$ and $Control_{sheep}$, but a greater O_2ER (p=0.010; Table 3) in comparison to the $Glipizide_{sheep}$ group. Additionally, the S_vO_2 was lower in the TEA_{sheep} group compared to $Glipzide_{sheep}$ (p=0.002)and $Control_{sheep}$ (p=0.011; Table 3).

Diuresis, plasma lactate and glucose were not different between groups during the entire time of the experiment (Table 3). Nevertheless, animals of the TEA_{sheep} group showed an increase in BE compared to $Control_{sheep}$ (p=0.005) and $Glipizide_{sheep}$ (p=0.002; Table 3).

Fluid infusion

There were no differences in the amount of crystalloid and colloid solutions per kilogram body weight and hours survival time when comparing groups of the sheep study (Table 4). In addition, the total volume infused per kilogram body weight and hours survival time between the groupsdid not differ between groups (Table 4).

Survival time

Animals in the TEA_{sheep} group had a reduced survival time (5.0 hours [4.1-5.9]) compared to $Control_{sheep}$ (9.0 hours [6.8-11.3]) with no animal reaching the end of the 12-hour intervention period (p=0.001; Figure 2). Average survival time did not differ between $Glipizide_{sheep}$ (8.0 hours [6.5-9.5]) and $Control_{sheep}$ (p=0.452). In addition, cumulative dosage of endotoxin did not differ between groups (Table 4).

Results of the rat study

There were no relevantbaseline differences in any of the investigated variables at the timepoint$Sepsis_{rat}$among the $Control_{rat}$, $Glipizide_{rat}$ and TEA_{rat} groups. The $Sham_{rat}$ group had a lower HR (Table 5).

Hemodynamic effects of K^+-channel inhibitors.

Infusion of glipizide resulted in an increase in MAP compared to $Sepsis_{rat}$measurement (p=0.012; Table 5and Supplemental figure 6, http://links.lww.com/SHK/A693). Both, glipizide and TEA, led to a reduction in HR (p<0.05 each; Table 5and Supplemental figure 6, http://links.lww.com/SHK/A693). RR did not change in any of the study groups (Table 5). In addition, all hemodynamic variables were stable over time in the $Sham_{rat}$ group (Table 5).

Effects of K^+-channel inhibitors on villi microcirculation.

Neither ICA_{total}, nor ICA_{cont} or the ratio ICA_{cont}/ ICA_{total} of the $Glipizide_{rat}$ group differed significantly from the $Sham_{rat}$ group or $Control_{rat}$ group (Table 6). However, the ICA_{total} and the ICA_{cont} were increased in the animals of the TEA_{rat} group compared to the $Sham_{rat}$ group (p=0.035 and p=0.002; Table 6). In addition, the ratio ICA_{cont}/ $ICA_{total}$was higher in the TEA_{rat} group compared to the $Glipizide_{rat}$ (p=0.013) and the $Sham_{rat}$ group (p=0.012; Table 6 and Figure 3).

Discussion

The major findings of the present study are thatin ovine endotoxemic shockcontinuous intravenous infusions of theK^+-channel inhibitors glipizide and TEA are not associated with a reduction in NE requirements compared to standard treatment with NE. Nevertheless,the selective K^+_{ATP}-inhibitor glipizide stabilized systemic hemodynamics (as indicated by an increased SVRI and a reduced HR), whereas the non-selective K^+-channel inhibitor TEA was associated with anincreased oxygen consumption and reduced survival time in ovine endotoxemic shock. Moreover, glipizide increased MAPwithout affecting the intestinal microcirculation in the rat septic shock model. Contrary, TEA resulted in a deterioration of rat villous microcirculationand exerted no statistical effects onMAP.

Administration of vasopressors in septic shock aims to maintain perfusion pressure by increasing SVRI and, thereby, to restore tissue perfusion. Microcirculatory alternations are frequent in sepsis, however, they do not necessarily correlate with macrohemodynamic changes(26). Because vasopressors carry the risks of excessive vasoconstriction and tissue hypoperfusion it is crucial to investigate their effects on the macro- and microcirculation(27). In the present study this was achieved by combining two models of endotoxemic and septicshock. Focusing on macrocirculatory parameters, previous trialsrevealed that intravenous bolus infusions of sulfonylureas, like glipizide, increased MAP and SVRI in experimental septic shock(17,28).However, experimental und clinical data suggest that bolus infusions of sulfonylureas in septic shock mediate no long-lasting effect on vascular tone(17,28,30,29).In this context, Lange et al. demonstrated that a continuous infusion of glipizide offers a longer-lasting effect on macrohemodynamicsin endotoxemic sheep(13).In the present study a continuous infusion of glipizide for 12h partially restored vasomotor tone in endotoxemic sheep, as indicated by an increase in SVRI. Nevertheless, the glipizideinfusionhad nosignificant effect on NE requirements. There are manifold potential explanations: It is known that the profound vasoplegia in septic shock is mediated not only by K^+_{ATP}-channels(12,32,31), but also by increases in NOproduction and potentially vasopressin deficiency, for example. Thus, selective K^+_{ATP}-channel inhibition alone might not always be sufficient to reduce NE requirements. On the other hand, it could be hypothesized that the guideline-oriented therapy with adequate fluid resuscitation masked potential differences in NE requirements. This assumption is supported by a tendencytowards lower NE requirements in the $Glipizide_{sheep}$ group, overall low NE requirements and large amounts of administered fluids in all three groups.

Contrary, TEA did not restore vascular tone in experimental endotoxemic and septic shock, but resulted in premature death of all TEA-treated sheep. Notably, TEA can induce neurotoxic effects by blocking autonomic ganglia. The observed reduction in HR in both studies after TEA infusion is likely due to its ganglion blocking properties(33). Although it lacks objective measurements, premortal animals in TEA_{sheep} group were agitated, a potential clinical sign of neurological toxicity. These unanticipated results stand in contrast to previously published studies, in which TEA counteracted sepsis-induced hypotension in small animals(32) or restored the vasopressor-response in human experimental endotoxemia and experimental sepsis in small animals(11,31).In this context, Sordi et al. previously reported the effects of two different TEA dosages in septic rats. A single low dose of TEA (8 $mg \cdot kg^{-1}$; SC) stabilized hemodynamics, whereas a higher dose (16 $mg \cdot kg^{-1}$; SC)was associated with an increased mortality(32).It can be speculated that the dose administered to sheep was excessive. However, previously performed dose-finding studies did not show the high mortality observed after 12-hours of TEA-infusion, which could be explained by ashorter observation period.

The infusion of glipizide was associated with a decrease in HR, CI, DO_2I and VO_2I in endotoxemic sheep.In addition to the findings of Lange et al., who attributed a similar observation to a baroreceptor reflex following an increase in SVRI(13), a significant decrease in SVI occurred in glipizide-treated sheep. It is most likely that the reduction in SVI is due to an elevation in SVRI, which may represent an increased left ventricular afterload. Notably, decreases in CI and DO_2I may compromise cellular oxygenation when falling below critical values. However, values of CI were supraphysiologic in all groups after supportive treatment with fluids and NE. Moreover, no signs of tissue hypoperfusion were observed, as indicated by BE, lactate or S_vO_2, which were all maintained.As a sulfonylurea, glipizide may have the ability

to decrease blood glucose concentrations and hypoglycemia is known to be deleterious in critical illness. However, the current study found no statistical differences between the groups of the sheep study in blood glucose concentrations during the experimental period.Taken together,no detrimental or unexpected effects of glipizidein the rat and sheep study were found.

In the present sheep study, TEA was associated with an increased oxygen consumption and a drop in S_vO_2. This may suggests an elevated oxygen demand, which is potentially deleterious in septic shock, where the oxygen utilization is *per se* impaired (34).

One relevant aspect of septic shock is that hypoperfusion of the intestinal microcirculation may contribute to translocation of bacteria, proteases, and toxins from the gut to the systemic circulation via breakdown of the gut barrier function. This may facilitate remote systemic complications in critically ill patients(35). This intestinal hypoperfusion may be aggravated by exogenous vasoconstrictors, which are commonly used in septic shock patients. In the present study, glipizide infusion increased MAP in septic rats, without compromising intestinal microcirculatory perfusion variables. These results challenge a previous report from Beck et al., who examined the effect of the sulfonylurea glibenclamide in septic rats. The latter study did not describe any effects of a single glibenclamide boluson macro- and microcirculatory variables(36). However, the dose of glibenclamide was much lower than the respective dose of glipizide in the current study and, thus, may not be sufficient to increase MAP in rats. In contrast,TEA infusion resulted in adeterioration of rat villi microcirculation, with more than 50% increase in ICA_{cont}, corresponding to a decreased capillary perfusion. It is likely that the increase in ICA_{cont} is due toedemaformation rather than an excessive vasoconstriction. This is supported by the fact that neither $D_{terminal}$ and V_{RBC} nor MAP exerted a significant change associated with TEA infusion. In addition, animals of the TEA_{rat} group showed macroscopically pronounced

ascites and bowl edema. This observation is in accordance with toxicity studies describing the development of edema after lethal and sublethal doses of TEA(37).

Limitations of the study. Apotential limitation of the sheep study is the initiationof shockbyendotoxin infusionwhich does not entirelyreflect human sepsis.However,due to its excellent standardizationandthe similarity tohemodynamicchangesinhuman vasodilatory shockit representsan internationally establishedmodel(14,17,13,38).A further limitation of the sheep study is that HES solutionswere used for fluid resuscitation, which arecontra-indicated at present in critically ill patients due to suspected increasesin renal failure(4,5).Notably, fluid therapy was based on the Surviving Sepsis Campaign guidelines, which were applicable at the time of study conduction andrecommended HES solutions as well as the use of static preload variablesin septic shock(19,18).Since fluid resuscitation was performed with the same protocol in all study groups, it should not impact on the effects of the study drugs.When interpreting the present data, it is important to note, that the pathophysiology of ovine endotoxemic shock and septic shock in rats differ significantly. A direct comparison of both models may be not valid, and, therefore, both studies should be viewed rather complementary than comparative.Moreover, the study drug doses were markedly different between the rat and the sheep study (especially the TEA dose). However, doses were derived from the published literature on this drug in the respective species. Nevertheless, it may be hypothesized that the TEA dose in rats was relatively high, which may have facilitated the negative effects on gut microvascular perfusion.Finally, microcirculatory perfusion in sepsis depends on multiple factors like volume therapy, or origin of sepsis, and, thus, an intact intestinal microcirculation not inevitably indicates the global absence of microcirculatory disturbances in the individual case.

Conclusion

This study demonstrates that selective inhibition of K^+_{ATP}-channels in vasodilatory shock by continuous infusion of glipizide couldpartially restorevasomotor tone in endotoxemic sheepand improve systemic hemodynamics in septic rats without exerting harmful effects on intestinal microcirculation. In contrast, non-selective K^+-channel inhibition by TEAfailed to exert beneficial effects on systemic macro- and micro-hemodynamicsin alarge animal modelofendotoxemicshock and in septicrats.In contrast, TEAdecreased average survival time in endotoxemicsheep andresulted in a deterioration ofvillous microcirculation in septic rats. Furtherexperimental studies, evaluating the effectsand the optimal treatment regime of selective K^+-channel inhibitorsmay be warranted to identify potential settings in which glipizide may improve relevant outcome variables.

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Figure legends

Figure 1 Experimental protocol of the sheep and rat study.

Figure 1 Experimental protocol of the sheep and rat study

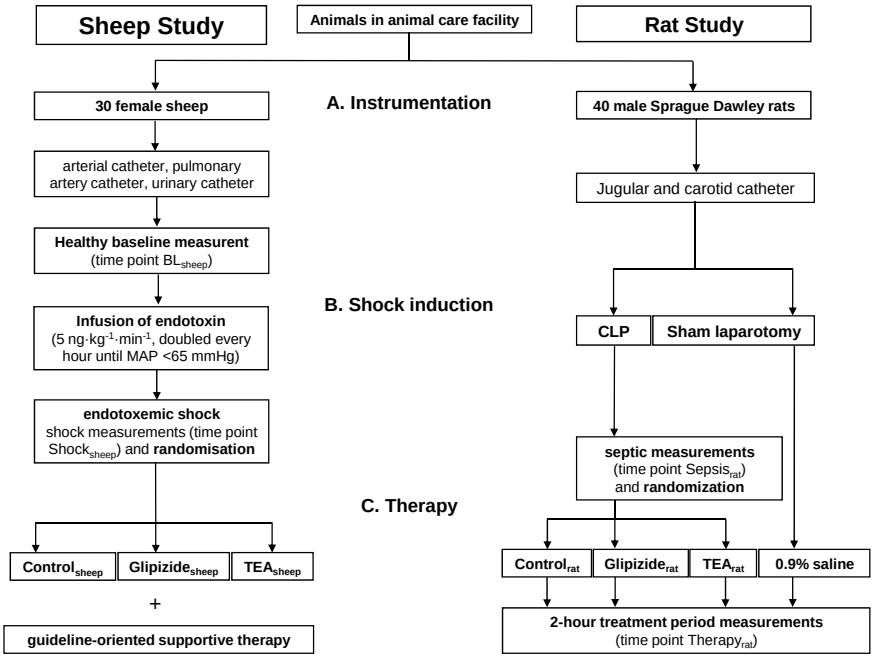


Figure 2 **Kaplan-Meier survival curves (sheep study).**

The TEA_{sheep} group had a significantly (*) reduced survival (5.0 hours [4.1-5.9]) compared to the $Control_{sheep}$ group (9.0 hours [6.8-11.3]; p=0.001). Survival time did not differ between $Glipizide_{sheep}$ (8.0 hours [6.5-9.6]) and $Control_{sheep}$ group.

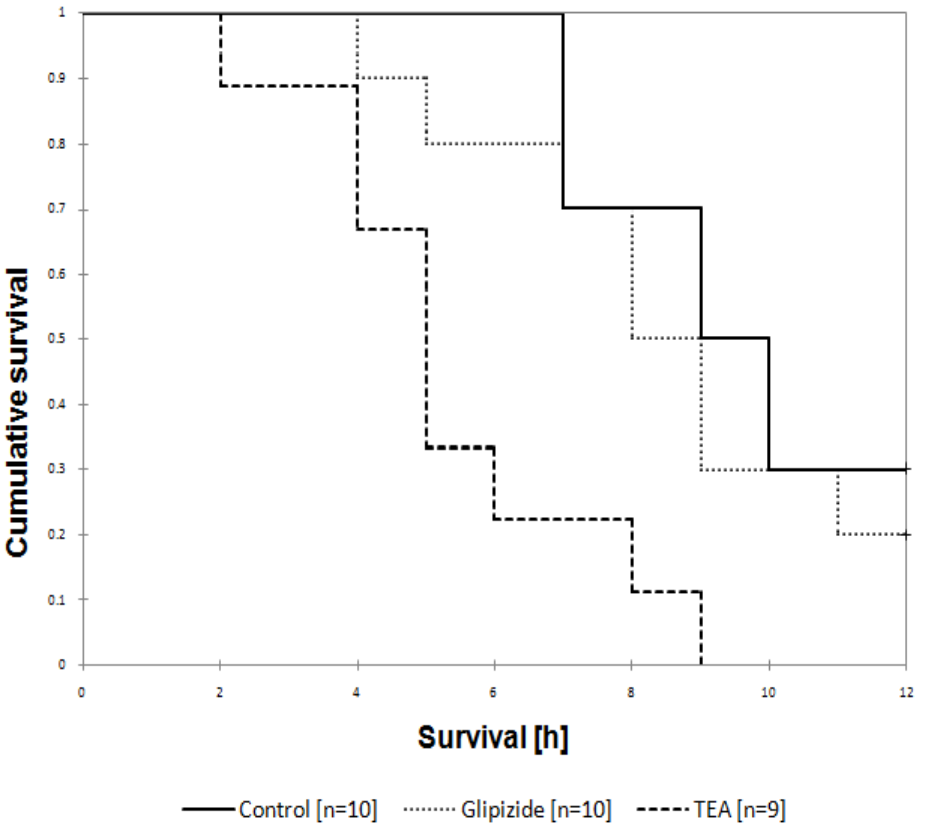
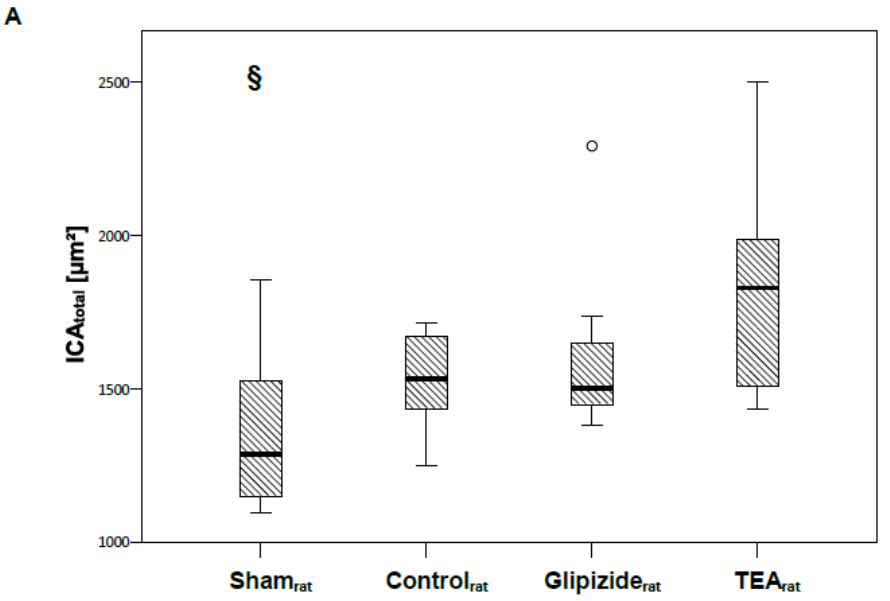


Figure 3 **Effects of K^+-channel inhibitors on the intestinal microcirculation after the two-hour intervention period (rat study).**

Data arepresentedas box plots. Intercapillary area size (ICA_{total}; A), Intercapillary area size of continuously perfused capillaries (ICA_{cont}; B), Ratio of intercapillary area size of continuously perfused capillaries and total intercapillary area size (ratio ICA_{cont}/ ICA_{total}; C). § indicates a significant difference ($p<0.05$) vs. TEA_{rat}; ○ indicates a statistical outlier in the presentation of the data as box plots.



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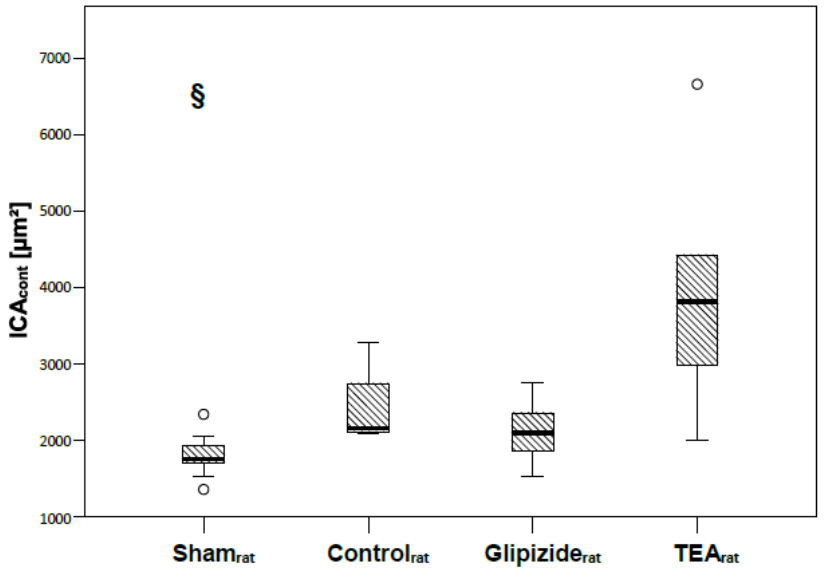

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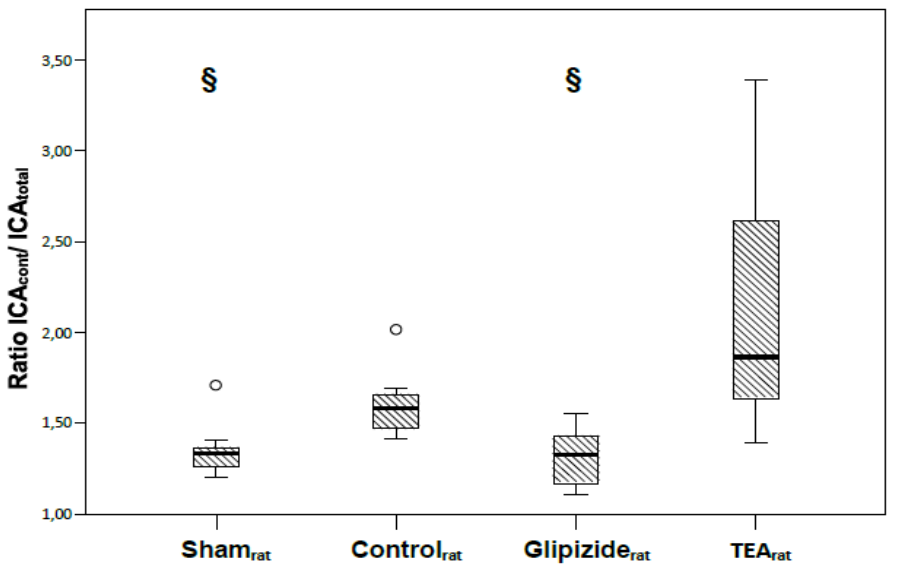

Table 1

Hemodynamics, variables of oxygen transport, metabolism and organ function at baseline and shock (sheep study).

Variable [Unit]	**BL_{sheep}**	**$Shock_{sheep}$**	***P* Value**
Hemodynamics			
HR [beat·min^{-1}]	90 [80; 98]	120 [106; 132]	<0.001 *
MAP [mmHg]	89 [86; 93]	64 [63; 65]	<0.001 *
SVRI [dyne·s·cm^{-5}·m^{-2}]	1255 [1061; 1519]	1096 [896; 1292]	0.008 *
SVI [mL·beats^{-1}·m^{-2}]	61 [55; 65]	38 [32; 47]	<0.001 *
CI [L·min^{-1}·m^{-2}]	5.2 [4.6; 6.1]	4.5 [4.0; 5.6]	0.041 *
CVP [mmHg]	5 [4; 9]	3 [2; 4]	0.002 *
LVSWI [g·m^{-1}·m^{2}]	67 [63; 72]	30 [26; 34]	<0.001 *
RVSWI [g·m^{-1}·m^{2}]	8 [7; 10]	9 [7; 10]	0.397
MPAP [mmHg]	16 [15; 18]	20 [17; 24]	0.001 *
PVRI [dyne·s·cm^{-5}·m^{-2}]	129 [81; 156]	204 [140; 316]	<0.001 *
PAOP [mmHg]	8 [6; 11]	8 [4; 12]	0.990
Oxygen transport variables			
Hct [%]	30 [27; 35]	35 [33; 41]	<0.001 *
S_vO_2 [%]	68 [63; 69]	67 [61; 70]	0.462
DO_2I [mL·min^{-1}·m^{-2}]	663 [569; 771]	701 [616; 774]	0.888
VO_2I [mL·min^{-1}·m^{-2}]	210 [164; 241]	205 [157; 220]	0.098
O_2ER [%]	30 [27; 35]	28 [25; 34]	0.127
Metabolic variables and organ function			
BE [mmol·L^{-1}]	2.2 [1.3; 3.2]	0.5 [-1.8; 1.7]	<0.001 *
pH [-log$_{10}$(H^+)]	7.46 [7.43; 7.50]	7.42 [7.38; 7.46]	0.001 *
Lactate [mmol·L^{-1}]	0.8 [0.6; 0.9]	2.9 [2.4; 4.0]	<0.001 *
P_aO_2/F_iO_2 [mmHg]	491.4 [475.2; 518.1]	489.5 [445.7; 511.4]	0.032 *
Temperature [°C]	39.7 [39.5; 39.7]	41.2 [40.8; 41.6]	<0.001 *
Glucose [mg·dL^{-1}]	78 [71; 89]	91 [81; 96]	0.011 *
Diuresis [mL·h^{-1}]	56 [40; 65]	27 [12; 44]	0.003 *

Data are presented as median [interquartile range]. Paired-samples Wilcoxon test was used to compare variables between BL_{sheep} and $Shock_{sheep}$; * indicates a statistical significant difference ($p<0.05$).

Abbreviation: BE, base excess; CI, cardiac index; CVP, central venous pressure; DO_2I, oxygen delivery index; Hct, hematocrit; HR, heart rate; LVSWI, left ventricle stroke work index; MAP, mean arterial pressure; MPAP, mean pulmonary arterial pressure; O_2ER, oxygen extraction ratio; PAOP, pulmonary artery occlusion pressure; pH, potentiahydrogenii; P_aO_2/F_iO_2, oxygenation index (Horowitz); PVRI, pulmonary vascular resistance index; RVSWI, right ventricle stroke work index; S_vO_2,mixed venous oxygen saturation; SVI, stroke volume index; SVRI, systemic vascular resistance index; VO_2I, oxygen consumption index.

Table 2

Differences of hemodynamic variables among groups (sheep study).

Variable [Unit]	**Group**	**Variation vs. Control$_{sheep}$**	**Variation vs. Glipizide$_{sheep}$**
HR [beat·min^{-1}]	Glipizide$_{sheep}$	-11 [-19; -2] *	-
	TEA$_{sheep}$	-13 [-24; -2] *	-2 [-12; 8]
MAP [mmHg]	Glipizide$_{sheep}$	2 [-3; 8]	-
	TEA$_{sheep}$	-3 [-9; 4]	-5 [-11; 1]
SVRI [dyne·s·cm^{-5}·m^{-2}]	Glipizide$_{sheep}$	246 [99; 394] *	-
	TEA$_{sheep}$	85 [-68; 238]	-161 [-346; 23]
SVI [mL·beats^{-1}·m^{-2}]	Glipizide$_{sheep}$	-8 [-15; 0] *	-
	TEA$_{sheep}$	0 [-8; 8]	7 [-1; 15]
CI [L·min^{-1}·m^{-2}]	Glipizide$_{sheep}$	-1.49 [-2.55; -0.42] *	-
	TEA$_{sheep}$	-0.85 [-1.91; -0.22]	0.64 [-0.33; 1.6]
CVP [mmHg]	Glipizide$_{sheep}$	-2 [-5; 0] *	-
	TEA$_{sheep}$	-1 [-3; 2]	2 [-1; 4]
LVSWI [g·m^{-1}·m^{2}]	Glipizide$_{sheep}$	-3 [-10; 4]	-
	TEA$_{sheep}$	0 [-8; 7]	3 [-4; 10]
RVSWI [g·m^{-1}·m^{2}]	Glipizide$_{sheep}$	1 [-2; 4]	-
	TEA$_{sheep}$	3 [1; 6] *	2 [-1; 5]
MPAP [mmHg]	Glipizide$_{sheep}$	0 [-4; 5]	-
	TEA$_{sheep}$	3 [-2; 7]	3 [-2; 7]
PVRI [dyne·s·cm^{-5}·m^{-2}]	Glipizide$_{sheep}$	47 [-116; 109]	-
	TEA$_{sheep}$	51 [-22; 125]	5 [-70; 79]
PAOP [mmHg]	Glipizide$_{sheep}$	0 [-3; 2]	-
	TEA$_{sheep}$	1 [-2; 3]	1 [-2; 4]

Data are presented as average difference vs. control during 12-hour observation [CI95%]. Multivariate analysis using generalized estimating equations were used to compare variables of interest; with dependent variable: variable of interest; independent variables: time (1 hour to 12 hours after Shock$_{sheep}$), dose of norepinephrine [µg·kg^{-1}·min^{-1}], study group, value of variable of

interest at BL_{sheep}. * indicates a significant difference ($p<0.05$) vs. $Control_{sheep}$; $^{\#}$ indicates a significant difference ($p<0.05$) vs. $Glipizide_{sheep}$.

Abbreviations: CI, cardiac index; CVP, central venous pressure; HR, heart rate; LVSWI, left ventricle stroke work index; MAP, mean arterial pressure; MPAP, mean pulmonary artery pressure; PAOP, pulmonary artery occlusion pressure; PVRI, pulmonary vascular resistance index; RVSWI, right ventricle stroke work index; SVI, stroke volume index; SVRI, systemic vascular resistance index.

Table 3

Differences of oxygen transport variables, metabolic variables, organ function and infusion rate of norepinephrine among groups (sheep study).

Variable [Unit]	Group	Variation vs. $Control_{sheep}$	Variation vs. $Glipizide_{sheep}$
Hct [%]	$Glipizide_{sheep}$	0 [-3; 3]	-
	TEA_{sheep}	0 [-3; 3]	0 [-3; 3]
S_vO_2 [%]	$Glipizide_{sheep}$	2 [-4; 7]	-
	TEA_{sheep}	-9 [-15; -2] *	-10 [-17; -4] #
DO_2I [mL·min^{-1}·m^{-2}]	$Glipizide_{sheep}$	-221 [-380; -61] *	-
	TEA_{sheep}	-142 [-327; 43]	79 [-63; 221]
VO_2I [mL·min^{-1}·m^{-2}]	$Glipizide_{sheep}$	-74 [-122; -25] *	-
	TEA_{sheep}	-3 [-57; 51]	71 [21; 120]#
O_2ER [%]	$Glipizide_{sheep}$	-4 [-9; 2]	-
	TEA_{sheep}	6 [-1; 12]	9 [2; 16]#
BE [mmol·L^{-1}]	$Glipizide_{sheep}$	-0.3 [-3.0; 2.4]	-
	TEA_{sheep}	3.4 [1.1; 5.8] *	3.7 [0.8; 6.6]#
pH [$-\log_{10}(H^+)$]	$Glipizide_{sheep}$	-0.01 [-0.06; 0.03]	-
	TEA_{sheep}	0.00 [-0.05; 0.05]	0.02 [-0.03; 0.06]
Lactate [mmol·L^{-1}]	$Glipizide_{sheep}$	0.3 [-1.0; 1.6]	-
	TEA_{sheep}	-0.5 [-1.8; 0.7]	-0.8 [-1.7; 0.0]
P_aO_2/F_iO_2 [mmHg]	$Glipizide_{sheep}$	-13 [-52; 26]	-
	TEA_{sheep}	-17 [-58; 23]	-4 [-55; 47]
Temperature [°C]	$Glipizide_{sheep}$	-0.3 [0.7; 0.1]	-
	TEA_{sheep}	-0.6 [-1; -0.1]*	-0.3 [-0.7; 0.2]
Glucose [mg·dL^{-1}]	$Glipizide_{sheep}$	5.6 [-5.0; 16.2]	-
	TEA_{sheep}	0.6 [-8.2; 9.4]	-5.0 [-14.6; 4.6]
Diuresis [mL·h^{-1}]	$Glipizide_{sheep}$	81 [-29; 190]	-
	TEA_{sheep}	46 [-86; 177]	-35 [-194; 124]
Norepinephrine infusion [µg·kg^{-1}·min^{-1}]	$Glipizide_{sheep}$	-0.266 [-0.548; 0.015]	-
	TEA_{sheep}	-0.084 [-0.380;	0.182 [-0.033; 0.397]

0.212]

Data are presented as average difference vs. control during 12-hour observation [CI 95%]. Multivariate analysis using generalized estimating equations were used to compare variables of interest; with dependent variable: variable of interest; independent variables: time (1 hour to 12 hours after $Shock_{sheep}$), dose of norepinephrine [$\mu g \cdot kg^{-1} \cdot min^{-1}$], study group, value of variable of interest at $Baseline_{sheep}$. * indicates a significant difference ($p<0.05$) vs. $Control_{sheep}$; $^{\#}$ indicates a significant difference ($p<0.05$) vs. $Glipizide_{sheep}$.

Abbreviations: BE, base excess; DO_2I, oxygen delivery index; Hct, hematocrit; O_2ER, oxygen extraction ratio; P_aO_2/F_iO_2, oxygenation index (Horowitz); pH, potentia hydrogenii; S_vO_2,mixed venous oxygen saturation; VO_2I, oxygen consumption index.

Table 4

Cumulative fluid infusion and cumulative norepinephrine and endotoxin dose (sheep study).

	Group		
Variable (Unit)	**Control$_{sheep}$**	**Glipizide$_{sheep}$**	**TEA$_{sheep}$**
Colloid ($mL \cdot kg^{-1} \cdot h^{-1}$)	3 [3; 4]	3 [3; 4]	4 [3; 4]
Crystalloid ($mL \cdot kg^{-1} \cdot h^{-1}$)	20 [16; 23]	20 [18; 26]	26 [18; 27]
Total volume infused ($mL \cdot kg^{-1} \cdot h^{-1}$)	27 [24; 30]	27 [25; 35]	35 [27; 39]
Norepinephrine($\mu g \cdot kg^{-1} \cdot h^{-1}$)	0.15 [0.09; 0.57]	0.06 [0.00; 0.08]	0.16 [0.00; 0.29]
Endotoxin ($mg \cdot kg^{-1} \cdot h^{-1}$)	0.08 [0.02; 0.12]	0.05 [0.02; 0.08]	0.04 [0.02; 0.05]
Glucose infusion ($mg \cdot kg^{-1} \cdot h^{-1}$)	0.9 [0.7; 1.1]	1.1 [1.0; 1.3]	1.1 [1.1; 1.4]

Values are presented as median [interquartile range] for each group.

Table 5

Hemodynamic variables before and after intervention (rat study).				
			Time point	
Group	**n**	**Variable [Unit]**	**$Sepsis_{rat}$**	**$Therapy_{rat}$**
$Sham_{rat}$	9	MAP [mmHg]	124 [114; 132]	125 [119; 129]
$Control_{rat}$	7		130 [123; 134]	131 [121; 133]
$Glipizide_{rat}$	8		119 [114; 139]	135 [130; 149] *
TEA_{rat}	7		118 [114; 127]	119 [113; 130]
$Sham_{rat}$	9	HR [beats·min^{-1}]	366 [348; 396] §	420 [348; 468]
$Control_{rat}$	7		418 [384; 480]	444 [348; 468]
$Glipizide_{rat}$	8		474 [444; 498]	432 [396; 486] *
TEA_{rat}	7		492 [462; 504]	408 [378; 456] #
$Sham_{rat}$	9	RR [breaths·min^{-1}]	95 [87;113]	90 [82;100]
$Control_{rat}$	7		112 [84; 150]	102 [64; 130]
$Glipizide_{rat}$	8		115 [87; 140]	113 [109; 144]
TEA_{rat}	7		140 [125; 152]	120 [103; 126]

Values are presented as median [interquartile range]. * $p<0.05$ vs. time point $Sepsis_{rat}$; § $p<0.05$ vs. time point $Sepsis_{rat}$ of the $Glipizide_{rat}$ group and vs.time point $Sepsis_{rat}$ of the TEA_{rat} group; # $p<0.05$ vs. time point $Sepsis_{rat}$.

Abbreviations: HR,heart rate; MAP, mean arterial pressure; RR, respiratory rate.

Table 6

Effect of K^+-channel inhibitors on villi microcirculation variables after the two-hour intervention period (rat study).

	Group			
Variable [Unit]	**$Sham_{rat}$**	**$Control_{rat}$**	**$Glipizide_{rat}$**	**TEA_{rat}**
ICA_{total} [μm^2]	1312 [1191; 1541]	1532 [1434; 1671]	1464 [1404; 1897]	1693 * [1564; 1828]
ICA_{cont} [μm^2]	1812 [1725; 1995]	2161 [2115; 2748]	2091 [1807; 2427]	4424 * [3212; 5541]
Ratio ICA_{cont}/ ICA_{total}	1.3 [1.3; 1.4]	1.6 [1.5; 1.7]	1.3 [1.2; 1.4]	2.6 *, § [2.0; 3.0]
$D_{terminal}$ [μm]	5.6 [5.0; 6.2]	5.2 [4.7; 6.0]	5.6 [4.5; 6.15]	5.4 [5.0; 5.9]
V_{RBC} [$\mu m \cdot min^{-1}$]	970 [742; 1364]	883 [674; 985]	775 [622; 964]	842 [717; 845]
$Q_{terminal}$ [$\mu L \cdot min^{-1}$]	23.6 [15.5; 31.3]	14.9 [12.0; 24.6]	19.4 [15.6; 21.7]	21.1 [17.6; 21.5]

Values are presented as median [interquartile range]. *$p<0.05$ vs. $Sham_{rat}$; §$p<0.05$ vs. $Glipizide_{rat}$.

Abbreviations: $D_{terminal}$,diameter of the terminal arteriole; ICA_{cont},intercapillary area size of continuously perfused capillaries; ICA_{total},intercapillary area size of all capillaries; $Q_{terminal}$,terminal arteriolar blood flow; V_{RBC},red blood cell velocity.