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In Vivo Evidence for Nitric Oxide–Mediated Calcium-Activated Potassium-Channel Activation During Human Endotoxemia

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Background—During septic shock, the vasoconstrictor response to norepinephrine is seriously blunted. Animal experiments suggest that hyperpolarization of smooth muscle cells by opening of potassium (K) channels underlies this phenomenon. In the present study, we examined whether K-channel blockers and/or nitric oxide (NO) synthase inhibition could restore norepinephrine sensitivity during experimental human endotoxemia.

Methods and Results—Volunteers received 2 ng/kg *Escherichia coli* endotoxin intravenously. Forearm blood flow (FBF) was measured with venous occlusion plethysmography. Infusion of 4 dose steps of norepinephrine into the brachial artery decreased the FBF ratio (ratio of FBF in the experimental arm to FBF in the control arm) to $84\pm4\%$, $70\pm4\%$, $55\pm4\%$, and $38\pm4\%$ (mean$\pm$SEM) of its baseline value. After endotoxin administration, norepinephrine-induced vasoconstriction was attenuated (FBF ratio, $101\pm4\%$, $92\pm4\%$, $83\pm6\%$, and $56\pm7\%$; n=30; $P=0.0018$; pooled data). Intrabrachial infusion of the K-channel blocker tetraethylammonium (TEA) completely restored the vasoconstrictor response to norepinephrine from $104\pm5\%$, $93\pm7\%$, $93\pm12\%$, and $69\pm12\%$ to $89\pm9\%$, $73\pm4\%$, $59\pm5\%$, and $46\pm8\%$ (n=6; $P=0.045$). Other K-channel blockers did not affect the response to norepinephrine. The NO synthase inhibitor N^G-monomethyl-L-arginine (L-NMMA; 0.2 mg $\cdot$ min^{-1} $\cdot$ dL^{-1} intra-arterially) also restored the norepinephrine sensitivity. In the presence of L-NMMA, TEA did not have an additional effect on the norepinephrine-induced vasoconstriction (n=6; $P=0.9$).

Conclusions—The K-channel blocker TEA restores the attenuated vasoconstrictor response to norepinephrine during experimental human endotoxemia. Coadministration of L-NMMA abolishes this potentiating effect of TEA, suggesting that NO mediates the endotoxin-induced effect on vascular K channels. In the absence of an effect of the selective adenosine triphosphate–dependent K-channel blocker tolbutamide, we conclude that the blunting effect of endotoxin on norepinephrine-induced vasoconstriction is caused by NO-mediated activation of calcium-activated K channels in the vascular wall. (*Circulation*. 2006;114:414-421.)

Key Words: inflammation ■ ion channels ■ nitric oxide synthase ■ pharmacology ■ regional blood flow ■ vasoconstriction

S epsis is the most common cause of death among patients in noncoronary intensive care units, resulting in an estimated mortality of 6 million patients each year worldwide.[1,2] Vasodilatory shock, defined as hemodynamic instability resulting from a decreased vascular tone and attenuated sensitivity to vasopressor therapy, is a characteristic feature of sepsis and the main cause of its mortality.[3]

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Increased synthesis of nitric oxide (NO) and activation of vascular potassium (K) channels[4] represent 2 pathways that appear to play an important role in the dysregulation of vascular tone in septic patients. Although it has been demonstrated that increased expression of inducible NO synthase is detectable only at the site of infection,[5] systemic administration of its inhibitor, N^G-monomethyl-L-arginine (L-NMMA), results in a rise in blood pressure and an increase in sensitivity to vasopressor drugs.[6]

Simultaneously, several inflammatory mediators may lead to activation of vascular K channels. Opening of vascular K channels hyperpolarizes the membrane of vascular smooth muscle cells, leading to closure of voltage-operated calcium channels and vasorelaxation. Moreover, activation of vascular K channels is associated with an attenuated response to vasoconstrictors,[7] and persistent membrane hyperpolarization by K-channel activation appears to account for much of the observed vascular hyporeactivity of septic shock.[7,8] Of the several subtypes of K channels present in the vascular wall, activation of the calcium-activated (K_{Ca}) and adenosine

Schedule of Measurements

Figure 1. Flow chart of the study protocol. The first norepinephrine dose-response curve represents the baseline norepinephrine sensitivity. The second (4 hours) includes the effect of LPS on norepinephrine sensitivity; the third (5 hours) shows the effect of the pharmacological intervention (placebo, K-channel blockers, and/or L-NMMA). HR indicates heart rate; MAP, mean arterial pressure.

triphosphate (ATP)–dependent (K_{ATP}) K channels are associated with inflammation-induced vascular hyporeactivity.

Animal experiments have demonstrated that administration of K-channel blockers during sepsis results in an increase in blood pressure, an increase in vasopressor sensitivity, and even a reduction in mortality.[9–12] Interestingly, NO is one of the mediators that activates the K_{Ca} channel during inflammation.[9,13,14]

Acute systemic inflammation can be induced by a low-dose infusion of *Escherichia coli* lipopolysaccharide (LPS) in healthy volunteers, resulting in changes in cardiovascular function comparable to those observed in sepsis.[15,16] Human data concerning the role of NO during inflammation are limited, and no human data are available concerning the role of vascular K-channel activation.

In the present study, we quantified the effect of LPS infusion on the vasoconstrictor response to norepinephrine in the human forearm vasculature in the absence and presence of K-channel blockade and NO synthase inhibition. The study addresses 3 questions: Is it possible to restore the vascular hyporeactivity to norepinephrine during human endotoxemia by pharmacological K-channel blockade? Which vascular K channel (K_{Ca} or K_{ATP}) is involved? And finally, is the activation of the vascular K channels during human endotoxemia mediated by NO?

Methods

Subjects

After approval was obtained from our ethics committee, 36 non-smoking subjects (18 women, 18 men) gave written informed consent to participate in the experiments. Subjects taking prescription drugs (except for oral contraceptives), aspirin, or other nonsteroidal antiinflammatory drugs were excluded. Screening of the subjects within 14 days before the test revealed no abnormalities in medical history and physical examination. Routine laboratory tests and ECG were normal. All subjects tested negative for HIV and hepatitis B. Ten hours before the experiment, subjects refrained from caffeine, alcohol, and food.

Study Design of Human Endotoxemia Experiments

After local anesthesia (lidocaine HCL 20 mg/mL), the brachial artery was cannulated with a 20-gauge catheter connected to an arterial pressure monitoring line (Siemens SC9000, Den Haag, The Nether-

lands). The arterial line was used to administer drugs (norepinephrine, K-channel blockers, L-NMMA), to monitor blood pressure, and to sample blood.

Heart rate and blood pressure monitoring started ≈2 hours before LPS administration and was continued until the end of the experiment. A second cannula was placed in a deep antecubital vein for prehydration (1.5 L of 2.5% glucose/0.45% saline solution in the hour before the administration of LPS) and LPS administration. All subjects received 150 mL/h 2.5% glucose/0.45% saline solution during the first 6 hours after the LPS administration and 75 mL/h until the end of the experiment.

At 0 hours, purified endotoxin (LPS) prepared from *E coli* O:113 was injected (2 ng/kg IV) in 1 minute. LPS in the current dose attenuates the vasoconstrictor response to adrenergic[16] and other vasoconstrictors.[17]

The course of body temperature was determined every 30 minutes for the first 6 hours after LPS administration with an infrared tympanic thermometer (Sherwood Medical, 's-Hertogenbosch, the Netherlands).

Laboratory Tests

Blood samples were taken to determine the time course and peak values per individual. Total leukocytes and C-reactive protein values were measured before LPS administration and at 1, 6, 12, and 22 hours after LPS with standard laboratory techniques. To determine the concentration of the various cytokines, plasma was processed immediately by centrifugation at $2000g$ at 4°C for 15 minutes and was stored at −80°C before analyses. Concentrations of tumor necrosis factor-α, interleukin (IL)-1β, IL-6, IL-10, and interferon-γ were determined in samples taken at baseline and at 60 and 90 minutes and 2, 3, and 4 hours after LPS administration with simultaneous Luminex assay.[18]

Forearm Blood Flow Measurements

Forearm blood flow (FBF) was determined in both forearms with venous occlusion plethysmography (Filtrass Domed, Munich, Germany) as previously described.[19,20] Briefly, venous occlusion was achieved by inflating the upper arm cuffs to 45 mm Hg. Strain gauges were placed on the forearms and connected to plethysmographs to measure changes in forearm volume in response to inflation of the venous-congesting cuffs. FBF and drug administration were normalized to forearm volume as measured with the water displacement method and expressed in milliliters per minute per deciliter forearm volume ($mL \cdot min^{-1} \cdot dL^{-1}$).

Norepinephrine Dose Response

After instrumentation of both arms and an equilibration period of 30 minutes, the vasoconstrictor response to infusion of norepinephrine

into the brachial artery was quantified by measuring FBF, as illustrated in Figure 1. Each dose-response curve started with a 5-minute period of baseline measurements, followed by intra-arterial norepinephrine infusion at 1 to 3 $ng \cdot min^{-1} \cdot dL^{-1}$ to 10 to 30 $ng \cdot min^{-1} \cdot dL^{-1}$ (5 minutes per dose).

Four hours after LPS administration, the norepinephrine dose-response curve was repeated to determine the LPS-induced effects on norepinephrine sensitivity. Thereafter, at 5 hours, without selection or formal randomization, one of the following protocols was applied.

Protocol 1: Time Control Experiments With Norepinephrine During Endotoxemia

Time control experiments were carried out in 6 subjects to investigate the repeatability of the vasopressor response to norepinephrine. To this end, the norepinephrine dose-response curve was repeated once more at 5 hours with NaCl 0.9% as the "placebo" pharmacological intervention.

Protocol 2: Effect of Various K-Channel Blockers on Norepinephrine Sensitivity During Endotoxemia

We used the K-channel blockers quinine, tetraethylammonium (TEA), and tolbutamide to investigate the role of K_{Ca} and K_{ATP} channel activation. Using the perfused forearm technique, we have previously demonstrated that these blockers inhibit the vasodilator response to activation of K_{Ca}[21] and K_{ATP}[22] channels.

In 3 groups of 6 subjects, the norepinephrine dose-response curve was repeated at 5 hours during continued intra-arterial infusion of TEA (1 $mg \cdot min^{-1} \cdot dL^{-1}$), quinine (50 $\mu g \cdot min^{-1} \cdot dL^{-1}$), or the selective K_{ATP} blocker tolbutamide (1 $mg \cdot min^{-1} \cdot dL^{-1}$).

Protocol 3: Effects of NO Synthase Inhibitor L-NMMA and Coinfusion of K-Channel Blocker TEA on Norepinephrine Sensitivity During Endotoxemia

To determine whether the effect of TEA was induced through a direct effect of LPS/cytokines on the vascular K channels or whether this effect was mediated by NO, we infused the NO synthase inhibitor L-NMMA (0.2 $mg \cdot min^{-1} \cdot dL^{-1}$) intra-arterially in another 6 subjects at 5 hours. After 5 minutes of infusion, the effects of L-NMMA on FBF were determined. During continued L-NMMA infusion, a norepinephrine dose-response curve was determined as described above.

In this group, the experiment was extended to study the effect of combining TEA and L-NMMA. After a 30-minute washout period (6 hours), L-NMMA and TEA were coinfused to assess whether there is an additional effect of K-channel blockade during NO synthase inhibition.

Protocol 4: Effect of K-Channel Blockers and NO Inhibition on Norepinephrine Sensitivity in the Absence of Endotoxemia

To assess the effect of intra-arterial administration of TEA and L-NMMA on the norepinephrine dose-response curve, 6 experiments without LPS administration were conducted. Study design was identical to that of the endotoxemia experiments. Because of the long half-life of L-NMMA, a crossover design could not be applied; therefore, L-NMMA always was infused last.

A norepinephrine dose-response curve was determined using the same method as in the endotoxemia experiments. After 30 minutes, intra-arterial TEA infusion (1 $mg \cdot min^{-1} \cdot dL^{-1}$) was started, and its effects on FBF were assessed. During continued TEA infusion, the norepinephrine dose-response was repeated. After another 30-minute washout period, intra-arterial L-NMMA infusion (0.2 $mg \cdot min^{-1} \cdot dL^{-1}$) was started. After determining the FBF response to L-NMMA, we repeated the norepinephrine dose-response curve as described above.

Drugs and Solutions

All solutions were freshly prepared on the day of the experiment. Endotoxin (US Standard Reference Endotoxin *E coli* O:113, USP, Rockville, Md), norepinephrine (1 mg/mL, Centrafarm, Etten-Leur, the Netherlands), quinine dihydrochloride (300 mg/mL, Dagra Pharma, Diemen, the Netherlands), tolbutamide (10 mg/mL, Clinical

Pharmacy, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands), and L-NMMA (100 mg ampoules, Clinalfa, Laufenflingen, Switzerland) were all dissolved in 0.9% NaCl. On the day of the experiment, TEA was reconstituted from a sterile powder (Sigma, St Louis, Mo), diluted in NaCl 0.9% to a concentration of 1 mg/mL, and passed through a 0.22-μm Millipore (Millipore, Milford, Mass) filter.

Data Analysis, Calculations, and Statistics

Measurement of the FBF in the noninfused arm was used as a contemporaneous time control for the infused arm so that systemic effects could be observed and vasoactive effects could be expressed as a quotient of the infused and the noninfused arm. According to the literature, the FBF ratio is the optimal approach to analyze data from this kind of research.[23] Baseline infused-to-noninfused forearm ratio was defined at 100%.

The effects of quinine, TEA, tolbutamide, L-NMMA, and coinfusion of L-NMMA and TEA are expressed in absolute FBF ($mL \cdot min^{-1} \cdot dL^{-1}$). Norepinephrine-induced changes in FBF are expressed as percent flow change. FBF data are the mean of the measurements obtained during the last 2 minutes of each infusion period (steady state).

The mean arterial pressure and heart rate were sampled every 5 minutes throughout the experiments. To determine the systemic effects of the LPS infusion, the group averages before the start of baseline measurements and the group averages at 4 hours are expressed.

The primary end point of this study is the effect of pharmacological interventions (K-channel blockade and NO inhibition) on norepinephrine-induced vasoconstriction in the human forearm vasculature during systemic inflammation. Using NQuery 6.0 (Statistical Solutions Ltd, Cork, Ireland), we calculated sample size for a univariate 1-way repeated-measures ANOVA with constant correlation. The computer program was used with a number of levels of 4 (norepinephrine infusion rates), an estimated standard deviation of 20% with a correlation of 0.5, a desired power of 80%, and an estimated standard deviation of 10% to 15% of the expected means of differences. With this approach, sample sizes of 4 to 7 were needed to demonstrate a difference between the repeated measures before compared with after intervention. Therefore, we chose to include 6 individuals in each group. LPS-induced changes in clinical, inflammatory, and hemodynamic parameters were determined to demonstrate that the inflammatory stimulus was adequate.

Shapiro-Wilk and Kolmogorov-Smirnov tests indicated a normal distribution of data. LPS-induced effects (pooled n=30) and the effects of all pharmacological interventions in the forearm were tested for significance with repeated-measures ANOVA. Paired data (within subjects, comparison of before and after intervention) were assessed with the Student t test. All data are expressed as mean±SEM unless otherwise specified. A value of $P<0.05$ was considered statistically significant.

The authors had full access to the data and take full responsibility for their integrity. All authors have read and agree to the manuscript as written.

Results

Baseline Characteristics

Baseline characteristics are presented in Table 1. Apart from a lower blood pressure at baseline in the subgroup that did not receive LPS, there were no other imbalances between the groups.

Changes in Clinical, Inflammatory, and Hemodynamic Parameters

LPS administration induced the expected and transient flulike symptoms. Body temperature increased from 36.4±0.1°C to 38.0±0.1°C at 4 hours ($P<0.0001$, repeated-measures ANOVA), and white blood cell count decreased from 5.9±0.3 to 2.1±0.1×10^9/L at 1 hour after LPS administration, after which there was an increase to

TABLE 1.   Demographic Characteristics of the Study Groups.

Study Group	LPS-Controls	LPS-TEA	LPS-Quinine	LPS-Tolbutamide	LPS–L-NMMA–TEA	No LPS–L-NMMA–TEA
Sex, male/female	3/3	3/3	3/3	3/3	3/3	3/3
Age, y	23±2	23±4	22±2	21±1	21±2	20±1
Height, cm	173±9	173±10	179±13	177±7	179±7	173±10
Weight, kg	71.7±8.3	67.7±9.9	73.3±13.7	73.4±10.4	76.7±12.8	67.6±11.0
BMI, kg/m^2	24.0±1.2	22.6±2.0	22.9±2.2	23.5±2.3	23.9±2.9	22.4±2.1
Systolic blood pressure, mm Hg	129±8	120±10	130±16	136±14	137±12	112±14*
Diastolic blood pressure, mm Hg	72±9	68±6	74±9	73±7	74±6	59±11*
Heart rate, bpm	57±10	58±13	70±9	60±10	62±8	76±15
Forearm volume, mL	1067±246	942±258	992±225	979±158	1008±267	858±163
Total cholesterol, mmol/L	3.6±0.6	3.6±0.8	3.6±0.7	3.4±0.5	3.4±0.5	3.3±0.5
LDL cholesterol, mmol/L	2.10±0.47	2.18±0.75	2.21±0.47	2.06±0.51	2.07±0.60	1.99±0.47
HDL cholesterol, mmol/L	1.13±0.20	1.08±0.22	1.09±0.33	0.94±0.12	0.97±0.19	0.96±0.25
TG, mmol/L	0.80±0.51	0.74±0.27	0.73±0.20	0.84±0.44	0.78±0.16	0.79±0.39

BMI indicates body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein; and TG, triglycerides. Blood pressure was measured intra-arterially; heart rate was assessed by ECG. Data are mean±SD.

*Apart from a significantly lower baseline blood pressure in the no LPS–L-NMMA–TEA group, there were no baseline imbalances between groups.

13.4±0.6 and 13.7±0.6×10^9/L at 6 and 12 hours, respectively ($P<0.0001$, repeated-measures ANOVA). C-reactive protein was below the detection limit at baseline, increasing to 5.6±1.2 mg/dL at 6 hours, 23.0±1.8 mg/dL at 12 hours, and 35.1±2.4 mg/dL at 22 hours after the injection of LPS ($P<0.0001$, repeated-measures ANOVA). All plasma cytokine concentrations increased significantly after the administration of LPS (Figure 2).

The hemodynamic response to LPS is characterized by a significant decrease in blood pressure, rise in heart rate, and increase in FBF (Table 2). As illustrated in Figure 3, intrabrachial infusion of norepinephrine induced a dose-dependent vasoconstrictor effect that was impaired 4 hours after the administration of LPS ($P=0.0018$, repeated-measures ANOVA between dose-response curves; n=30).

## Protocol 1: Time Control Experiments With Norepinephrine During Endotoxemia

In this group, FBF was 4.0±0.8 before and 8.7±2.4 mL · min^{-1} · dL^{-1} after the administration of LPS. Figure 4A illustrates that with no pharmacological intervention the impaired vasoconstrictor response to norepinephrine is similar at 4 hours and 5 hours (P=nonsignificant [NS], repeated-measures ANOVA), indicating good repeatability of the impaired norepinephrine dose-response curve at 5 hours after the administration of LPS.

Protocol 2: Effect of Various K-Channel Blockers on Norepinephrine Sensitivity During Endotoxemia

In the quinine group, LPS increased FBF from 2.6±0.3 to 5.8±1.1 mL · min^{-1} · dL^{-1}. As illustrated in Figure 4B, quinine did not change the norepinephrine sensitivity (P=NS). In the tolbutamide group, baseline FBF averaged 2.3±0.3 mL · min^{-1} · dL^{-1}, which increased to 6.4±1.1 mL · min^{-1} · dL^{-1} after the administration of LPS. Intrabrachial infusion of the K$_{ATP}$ blocker tolbutamide had no effect on norepinephrine sensitivity (P=NS; Figure 4C). Both quinine and tolbutamide had no direct effect on FBF, blood pressure, or heart rate (data not shown).

Administration of LPS increased FBF from 2.6±0.5 to 5.8±1.3 mL · min^{-1} · dL^{-1} in the TEA group. Intrabrachial
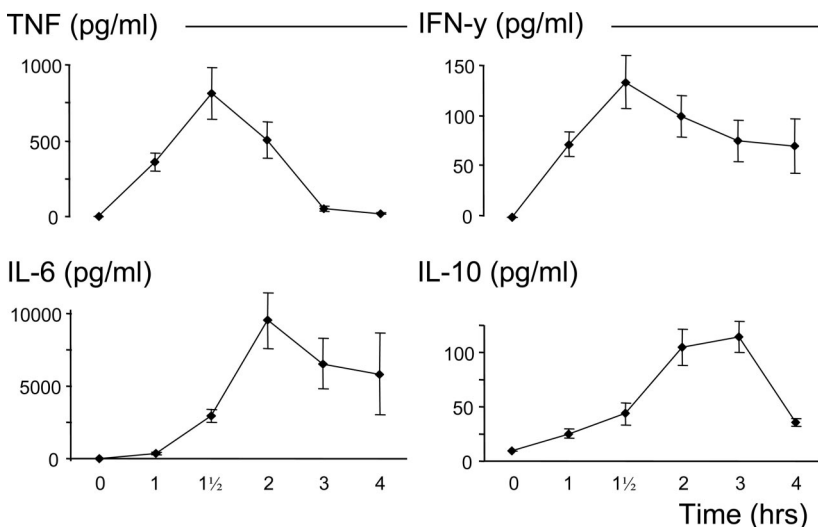
Figure 2. LPS-induced changes in cytokine plasma concentrations. Administration of 2 ng/kg *E coli* LPS resulted in a marked increase in tumor necrosis factor-α, IL-6, interferon-γ, and IL-10 concentrations. Data are expressed as mean±SEM (n=18). Changes in cytokine concentrations were all significant ($P<0.0001$) as analyzed with repeated-measures ANOVA over the complete curve. TNF indicates tumor necrosis factor; INF-y, interferon-γ.

TABLE 2.   Pooled Data of Cardiovascular and Hemodynamic Parameters of All LPS-Treated Subjects
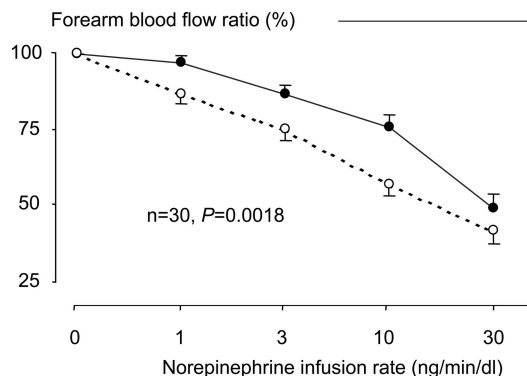
	Baseline	4 Hours After LPS	P
HR, bpm	67 ± 3	95 ± 2	<0.0001
SBP, mm Hg	145 ± 4	122 ± 3	<0.0001
DBP, mm Hg	79 ± 3	62 ± 2	<0.0001
FBF, mL · min^{-1} · dL	2.9 ± 0.3	7.0 ± 0.6	<0.0001
FVR, AU	39.0 ± 3.5	13.3 ± 1.2	<0.0001

HR indicates heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; FVR, forearm vascular resistance; and AU, arbitrary units. Administration of 2 ng/kg *E coli* LPS resulted in significant cardiovascular changes, illustrating that LPS induces a vasodilatory state. Data are expressed as mean± SEM of 30 experiments. The probability values refer to the difference between baseline and 4 hours after the administration of LPS as analyzed with repeated-measures ANOVA over the complete curves (HR, SBP, DBP) or Student *t* test for paired data (FBF and FVR). n=30.

administration of TEA at 4 hours after LPS administration did not change the FBF in the infused arm (from 5.8 ± 1.3 to 5.5 ± 1.7 mL · dL^{-1}; *P*=NS). Figure 5A shows the potentiating effect of TEA on the LPS-impaired norepinephrine dose-response curve (*P*=0.045, repeated-measures ANOVA). Intrabrachial administration of TEA did not significantly change the contralateral FBF (6.1 ± 1.0 to 5.7 ± 1.1 mL · min^{-1} · dL^{-1}), blood pressure ($109\pm4/59\pm2$ to $106\pm5/60\pm2$ mm Hg), or heart rate (92 ± 5 to 89 ± 5 bpm).

Protocol 3: Effects of NO Synthase Inhibitor L-NMMA and Coinfusion of K-Channel Blocker TEA on Norepinephrine Sensitivity During Endotoxemia

Figure 5B shows the norepinephrine dose-response curve in the presence of the NO synthase inhibitor L-NMMA. In this group, FBF increased from 2.8 ± 0.6 mL · min^{-1} · dL^{-1} at baseline to 6.3 ± 0.9 mL · min^{-1} · dL^{-1} after the administration of LPS. Intrabrachial administration of L-NMMA reduced FBF to 3.5 ± 0.8 mL · min^{-1} · dL^{-1} (*P*<0.005, paired *t* test), with no significant changes in the contralateral arm

Figure 3. Dose-response curve of intrabrachial norepinephrine (1 to 3 ng · min^{-1} · dL^{-1} to 10 to 30 ng · min^{-1} · dL^{-1}) on FBF before (open symbols, dotted line) and 4 hours after (solid symbols) administration of 2 ng/kg *E coli* LPS. Percentages of baseline FBF ratio (intervention/control arm) are presented as mean±SEM (n=30). LPS significantly attenuated the vasoconstrictor response to norepinephrine. The probability value refers to the statistical difference between the dose-response curves as analyzed with repeated-measures ANOVA.
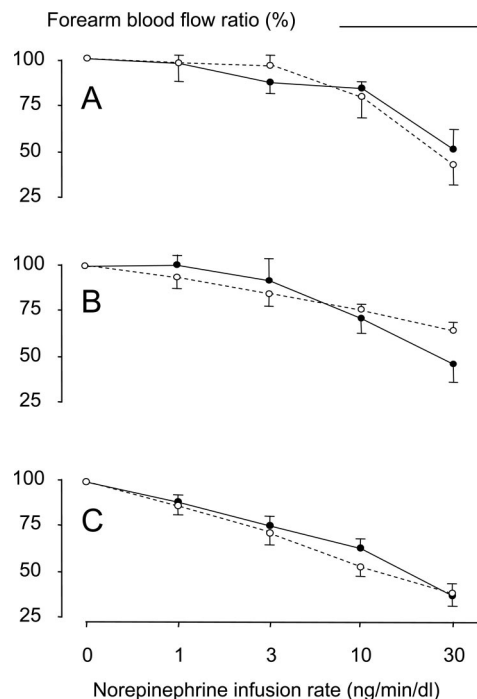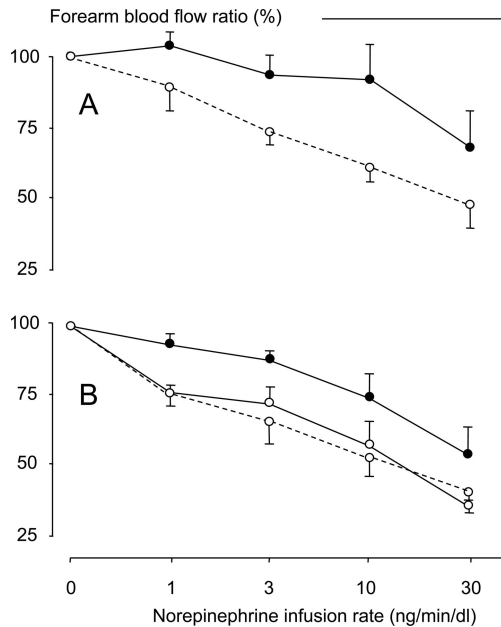

Figure 4. A, Percentage decrease in the ratio of the FBF in the infused and noninfused arms during graded intrabrachial norepinephrine infusion (1 to 3 ng · min^{-1} · dL^{-1} to 10 to 30 ng · min^{-1} · dL^{-1}) 4 hours after 2 ng/kg *E coli* LPS both in the absence (solid symbols) and presence (5 hours; open symbols, dotted line) of concomitant infusion of NaCl 0.9% (time controls, n=6; *P*=NS). B, The same protocol, now in the absence (solid symbols) and presence (open symbols) of concomitant infusion of quinine (50 μg · min^{-1} · dL^{-1}; *P*=NS). C, The same protocol, now in the absence (solid symbols) and presence (open symbols) of concomitant infusion of tolbutamide (1 mg · min^{-1} · dL^{-1}; *P*=NS). Data are presented as mean±SEM. The probability values refer to the statistical difference between the dose-response curves as analyzed by repeated-measures ANOVA.

(6.2 ± 1.1 to 5.8 ± 1.2 mL · min^{-1} · dL^{-1}), blood pressure ($126\pm4/67\pm2$ to $123\pm2/68\pm1$ mm Hg), or heart rate (92 ± 4 to 88 ± 4 bpm). L-NMMA significantly restored the attenuated vasoconstrictor response to norepinephrine (*P*=0.009, repeated-measures ANOVA). The subsequent norepinephrine dose-response curve in the presence of both L-NMMA and TEA demonstrated that TEA does not exert an additional potentiating effect in the presence of L-NMMA.

Protocol 4: Effect of K-Channel Blockers and NO Inhibition on Norepinephrine Sensitivity in the Absence of Endotoxemia

Norepinephrine (1 to 3 · min^{-1} · dL^{-1} to 10 to 30 ng · min^{-1} · dL^{-1}) -induced vasoconstriction was $98\pm11\%$, $86\pm11\%$, $56\pm9\%$, and $31\pm5\%$. Intrabrachial infusion of TEA did not change FBF (from 3.6 ± 0.7 to 2.8 ± 0.8 mL · min^{-1} · dL^{-1}; *P*=NS) and did not alter the sensitivity to norepinephrine (in the presence of TEA, $92\pm11\%$, $106\pm20\%$, $86\pm14\%$, and $52\pm15\%$; *P*=NS) in the absence of endotoxemia.

Intrabrachial infusion of L-NMMA decreased FBF from 3.3 ± 1.7 to 1.9 ± 0.6 mL · min^{-1} · dL^{-1} (*P*<0.01), with no significant changes in the FBF of the control forearm, blood pressure, and heart rate (data not shown). The norepineph-

Figure 5. A, Percentage decrease in the ratio of the FBF in the infused and noninfused arms during graded intrabrachial norepinephrine infusion (1 to 3 ng · min^{-1} · dL^{-1} to 10 to 30 ng · min^{-1} · dL^{-1}) 4 hours after 2 ng/kg *E coli* LPS both in the absence (solid symbols) and presence (5 hours; open symbols, dotted line) of concomitant infusion of TEA (1 mg · min^{-1} · dL^{-1}; n=6). B, The same protocol, now in the absence (solid symbols) and presence (open symbols) of concomitant infusion of L-NMMA (0.2 mg · min^{-1} · dL^{-1}; *P*=0.009) or L-NMMA+TEA (open symbols with dotted line; *P*=NS vs L-NMMA alone; n=6). Data are presented as mean±SEM. The probability values refer to the statistical difference between the dose-response curves as analyzed by repeated-measures ANOVA.

rine-induced vasoconstriction in the presence of L-NMMA also was not significantly different from the baseline dose-response curve (in the presence of L-NMMA, 84±14%, 56±9%, 50±10%, and 35±12%).

During the preparation of this manuscript, additional experiments with LPS and TEA using the same protocol as in the present study were conducted. In the group of 10 subjects, the probability value of the TEA-mediated improvement of the norepinephrine vasoconstriction was 0.015.

Discussion

Our study demonstrates that blockade of vascular K channels or inhibition of NO synthase restores the attenuated vasopressor effect of norepinephrine during human experimental endotoxemia. Furthermore, K-channel blockade in the presence of the NO inhibitor L-NMMA did not further potentiate the norepinephrine vasoconstrictive effects, suggesting that the endotoxin-induced effect on K channels is mediated by NO. Additional experiments with various K-channel blockers indicate that the effects of endotoxin on the vasculature are the result of NO-mediated opening of K_{Ca} channels.

Our findings on LPS-induced vasodilation and hyperactivity to norepinephrine confirm previous reports using the human endotoxemia model.[16,17] Pleiner et al[16] demonstrated that infusion of LPS produced a systemic hypotensive and inflammatory reaction that was associated with a reduced

sensitivity to adrenoceptor agonists. However, this profound reduction in adrenoceptor-mediated constriction was not associated with an altered effect of NO synthase inhibition because local L-NMMA administration did not lead to a more pronounced vasoconstriction during endotoxemia compared with control circumstances. In the present study, we found that intra-arterial administration of L-NMMA does potentiate the vasopressor response to norepinephrine. This may suggest that LPS-induced vasodilatation and the development of hypotension/vasodilation on one hand and the induction of vascular LPS-induced hyporeactivity to norepinephrine on the other hand may reflect different mechanisms. Our observation that NO inhibition does not potentiate the vasoconstrictor response to norepinephrine under control circumstances (in the absence of LPS) is consistent with a previous report.[24]

Of course, this observed effect of L-NMMA does not necessarily imply that NO synthase inhibition is beneficial in septic patients. Although systemic treatment of patients with septic shock resulted in a significant rise in blood pressure,[6] this was unfortunately associated with an increase in mortality in a randomized clinical trial.[25] Both the fact that L-NMMA is a nonselective NO synthase inhibitor that blocks inducible and endothelial NO synthase and the dosage of L-NMMA used in that clinical trial may have accounted for the observed detrimental effects.

K-Channel Activation During Inflammation

Persistent membrane hyperpolarization by K-channel activation appears to account for much of the observed vascular hyporeactivity of septic shock.[7,8] Charybdotoxin, a selective blocker of K_{Ca} channels, restores LPS-induced vascular hyporeactivity in isolated blood vessels, whereas glibenclamide, a selective antagonist of K_{ATP} channels, is not effective in vitro.[9,26] In contrast, K_{ATP} blockers increase blood pressure during experimental septic shock in various in vivo animal models.[11,12] Thus, from animal experiments, there is good evidence that K-channel blockers are beneficial in the treatment of vasodilatory shock, leading to an increase in blood pressure, an increase in vasopressor sensitivity, and even a reduction in mortality.[9–12] We have previously demonstrated that vasodilation associated with pharmacological K-channel activation by diazoxide is associated with a distinct blunting of the vasoconstrictor response to norepinephrine in humans.[22] In addition, we found that concurrent administration of the K-channel blocker TEA could reduce the diazoxide-mediated vasodilation, indicating that pharmacological modulation of vascular K channels may provide a novel treatment opportunity.[22] Nevertheless, until now, the role of K-channel activation during systemic inflammation in humans was unknown. Our results on the LPS-induced attenuated norepinephrine sensitivity and its restoration by TEA in humans are consistent with previous data showing full reversal of hyperpolarization[27] and hyporeactivity[27,28] by TEA in isolated vessels. Because TEA is a nonselective blocker of both K_{Ca} and K_{ATP} channels, the experiments with TEA do not differentiate between these 2 vascular K channels. Because the selective K_{ATP} blocker tolbutamide showed no potentiating effect, however, we have to conclude that vascular K_{Ca}

channels mediate the vascular hyporesponsiveness to norepinephrine during human endotoxemia. This observation is in accordance with various in vitro studies that demonstrate involvement of the K_{Ca} channel, and not the K_{ATP} channel, with the use of TEA[8,9] or highly specific K_{Ca} channel blockers like charybdotoxin[8,9] and iberiotoxin.[29] The absence of a potentiating effect of quinine may well be explained by its rather poor specificity because quinine also acts as an α-adrenergic blocking agent.[30] The fact that the vasoconstrictor response to various agonists (norepinephrine, angiotensin II, vasopressin) is attenuated during endotoxemia[17] supports the idea that the final common pathway is affected (influx of calcium and an increase in intracellular calcium), and not the specific receptors or specific signaling pathways for the aforementioned vasoconstrictor substances. Thus, it is likely that TEA also will improve the vasoconstrictor response to angiotensin II and vasopressin because the action of these agents is also dependent of calcium influx from voltage-operated Ca channels, of which the open-state probability is mediated predominantly by K channels. Moreover, our control experiments performed in subjects who did not receive LPS demonstrate that TEA does not potentiate the norepinephrine response in the absence of systemic inflammation, indicating that the potentiating effect of TEA is not due to a direct interaction with the vascular α-receptor.

The intracellular signaling by which K channels are activated during human endotoxemia is largely unknown. Because K-channel blockade with TEA did not exert an additional effect during NO synthase inhibition, NO may be responsible for the activation of the vascular K channels. Interestingly, apart from direct activation by high concentrations of LPS,[31] patch-clamp experiments have shown that NO can directly[13] and indirectly (through guanylate cyclase)[14] activate single vascular K_{Ca} channels. The NO-mediated pathway appears plausible because LPS is cleared from the bloodstream within 20 minutes during experimental human endotoxemia and because cytokines do not activate vascular K channels or directly dilate human vessels.[32]

Recently, Singer and colleagues[33] reported that life-threatening complications related to K-channel–opening drugs subsided after the administration of the K-channel blocker glibenclamide. Glibenclamide appeared to be effective in several cases, but not in septic shock. Our data confirm this observation of Singer et al that selective K_{ATP} blockade with sulfonylurea derivatives (tolbutamide, glibenclamide) may not be helpful in the hemodynamic management of septic shock. According to our data, other K-channel blockers like TEA may be more effective.

Therapeutic Implications
In the past, TEA was used as an antihypertensive drug because of its ganglion-blocking properties.[34] This effect is not confounding the data in the present study because we infused the drug into the brachial artery, distal of the sympathetic ganglia. On the basis of its ganglion-blocking properties, systemic administration of TEA may have a blood pressure–lowering effect in septic patients with an activated sympathetic system, which may limit its use in this specific group of patients. However, systemic administration of TEA

in normotensive patients did not lower blood pressure.[35] In experimental animals, systemic treatment with TEA during endotoxic shock in rats did not deteriorate the LPS-induced fall in blood pressure,[36] and intravenously administered TEA fully restored the responsiveness to phenylephrine in rats injected with LPS.[37] Although the data are limited, these results are encouraging and suggest that TEA may represent a possible future therapeutic opportunity to modulate vascular tone in septic shock patients. The next step to explore the potential therapeutic properties of K-channel blockers in vasodilatory shock would be systemic administration of TEA during experimental endotoxemia or in septic patients.

Conclusions
Our experiments indicate that NO-mediated activation of K_{Ca} channels is responsible for the observed hyporeactivity to norepinephrine during human endotoxemia and confirm in vitro and animal data that pharmacological inhibition of vascular K channels restores the attenuated norepinephrine sensitivity during systemic inflammation. Pharmacological modulation of vascular K channels represents a novel treatment modality for septic shock.

Disclosures
None.

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CLINICAL PERSPECTIVE

Worldwide, an estimated 6 million patients die of sepsis each year. Vasodilatory shock, characterized by systemic vasodilatation and an attenuated sensitivity to vasopressor therapy with norepinephrine, is the main cause of mortality during sepsis. Recent research indicates that the attenuated response to norepinephrine is caused by activation of vascular potassium (K) channels. The opening of these channels, triggered by inflammation and induction of nitric oxide, hyperpolarizes the vascular smooth muscle cell, leading to closure of voltage-operated calcium channels and a decrease in intracellular calcium. Consequently, the decreased intracellular calcium concentration results in an attenuated norepinephrine-induced smooth muscle contraction and vasoconstrictor response. This pathophysiological mechanism has been widely examined in various animal and in vitro models of inflammation, whereas human data are lacking. The present study demonstrates that administration of *Escherichia coli* lipopolysaccharide to healthy human volunteers induces a systemic inflammatory response, leading to a blunted vasoconstrictive response to norepinephrine in the human forearm vasculature. More importantly, it is demonstrated that coinfusion of the K-channel blocker tetraethylammonium completely restores the attenuated response to norepinephrine during systemic inflammation. This implies that in humans vascular K-channel activation plays a pivotal role in the reduced vasoconstrictor response to norepinephrine during systemic inflammation. Consequently, K-channel blockade could be a novel method to reduce sepsis-induced vasorelaxation and to improve the response to vasopressor therapy with norepinephrine. This finding warrants additional research into vascular K-channel blockade in septic patients to improve the management of hemodynamic instability in this group of patients.