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Vascular BK channel deficiency exacerbates organ damage and mortality in endotoxemic mice

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Abstract

We determined the contribution of vascular BK channels to endotoxin (lipopolysaccharide, LPS)-induced hypotension, organ damage, and mortality using smooth muscle BK channel deficiency (BK channel β1-subunit knockout, BK β1-KO) mice. BK β1-KO mice were more sensitive to LPS-induced mortality compared to wild-type mice. After LPS (20 mg/kg, intraperitoneally), BK β1-KO mice had a more rapid fall in heart rate and blood pressure (measured by radiotelemetry), shorter latency to mortality, and higher mortality rate than wild-type mice. Twenty-two hours after LPS treatment, wild-type and BK β1-KO mice had reduced norepinephrine reactivity and impaired constrictor responses to the BK channel blocker paxilline in mesenteric arteries *in vitro*; and higher iNOS expression in the heart, but not in mesenteric arteries. Endotoxemic BK β1-KO mice also showed more severe lung and intestinal injury, higher myeloperoxidase activity and polymorphonuclear neutrophil infiltration in lung and liver. Endotoxemic BK β1-KO mice had higher plasma tumor necrosis α and interleukin 6 levels at 22 hours, but not 6 hours post-LPS. Exaggerated mortality in BK β1-KO mice also occurred in the cecal ligation/puncture model of septic shock. Reduced vascular BK channel function does not protect against hypotension in the early stage of septic shock; in the later stage, smooth muscle BK channel deficiency enhances organ damage and mortality.

Keywords

BK β1-knockout; endotoxemia; hypotension; organ damage; mortality

Introduction

In the USA, septic shock kills 250,000 people annually, which is the second-leading cause of death in non-coronary ICU patients. Sepsis occurs when initially appropriate host immune

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None

responses to an infection become dysregulated. Although sepsis is not primarily a cardiovascular problem, ~50% of septic shock patients die from hemodynamic abnormalities within the first 24 hours postinfection, before antimicrobial therapies have time to become effective.[1–3] Septic shock causes hypotension, reduced blood flow to critical organs, multiple organ failure and death. Hypotension is caused mainly by insensitivity to endogenous norepinephrine (NE) via iNOS-derived nitric oxide (NO),[4] which reduces peripheral resistance and increases vascular capacitance thus causing peripheral blood pooling.

Large conductance Ca^{2+} activated K^+ channels (BK) channels are composed of pore forming α-subunits, and accessory β-subunits that modulate α-subunit Ca^{2+} sensitivity and channel activity.[5,6] In vascular smooth muscle cells, BK channels complex with L-type Ca^{2+} channels where BK channels function as negative-feedback modulators of vascular tone by regulating L-type Ca^{2+} channel activity.[5,6] Activation of BK channels causes vascular smooth muscle cell hyperpolarization, L-type Ca^{2+} channel closure and vasodilation.[5,6] BK channel activation prevents NE-induced arterial constriction *in vitro*.[7,8] Blockade of BK channels improves arterial reactivity to NE in humans and survival in endotoxemic mice,[9,10] and inhibits sepsis-induced arterial relaxation *in vitro*.[11,12,13] These data suggest that BK channels contribute to sepsis-induced vasodilation. Theoretically, BK channel blockers should protect against sepsis-induced vascular hypocontractility and prolonged hypotension.[7] Surprisingly, a recent study using a global BK channel α-subunit knockout (α-KO) mouse showed that BK channel deficiency does not protect against polymicrobial sepsis-induced hypotension and instead caused exaggerated mortality via an unknown mechanism.[14] These results are provocative, but use of mice with global BK channel deficiency prevented precise mechanistic interpretation because differences in sepsis-induced hypotension and mortality in the BK channel α-subunit KO mice could not be attributed to vascular changes only.[14] Phenotypic characteristics other than the absence of vascular BK channels might contribute to the higher mortality in septic BK α-KO mice; for example, these mice exhibit delayed organ development, sudden death, ataxia and deficits in motor performance.[14,15] Therefore, studies of BK channel function in septic shock in BK α-KO mice are complicated by the fact that BK channels are expressed by multiple tissues *in vivo*. These complications would be minimized in studies of mice with a specific SMC BK channel deficiency.

There are 4 subtypes (β1-β4) of BK channel β-subunits and β1-subunits are specific for SMC.[5, 16] BK β1-KO reduces the Ca^{2+} sensitivity of the pore-forming α-subunit, therefore, causing vascular SMC membrane depolarization, and increased vascular tone and NE reactivity.[5, 17, 18] Using BK β1-KO mice in shock studies would allow separation vascular from other tissues (nerves, kidney, immune system) responses that may be responsible for organ damage and hemodynamic regulation in septic shock. In addition, BK β1-KO mice have normal development and behavior[14, 15] and they are not hypertensive.[18] Therefore, we compared blood pressure, heart rate (HR) and mortality in lipopolysaccharide (LPS) treated BK β1-KO and wild-type (WT) mice. We also determined the possible contributions of vascular BK channels to sepsis-induced hypotension and mortality. Since LPS-induced hypotension does not mimic all pathophysiological changes seen in septic patients,[19] we

also tested mortality in septic shock caused by cecal ligation and puncture (CLP), a more realistic model for the induction of polymicrobial sepsis.

Material and Methods

Animals

Homozygous breeder male and female BK channel β1-KO mice were given to us by Dr. Robert Brenner (University of Texas Health Science Center, San Antonio, TX), and the mice were bred in the animal care facility at Michigan State University.[18] BK channel β1-KO mice are congenic as a result of seven generations of inbreeding to the C57BL/6 line and maintained originally as homozygous lines.[18,20] Control WT (C57BL/6) mice were from Charles River Laboratories. Pups of BK β1-KO mice were weaned at 3 weeks, and all mice were fed a normal diet. Mice used in our studies were at 10–12 weeks of age (male, 25–30g). All of the studies were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996) and approved by the Michigan State University Institutional Animal Care and Use Committees.

Measurements of MAP, HR and survival

Procedures used for telemeter implantation in mice have been described.[18] Under isoflurane anesthesia (2%–3%), a catheter attached to a radiotelemetry transmitter (Data Sciences International, St. Paul, MN) was inserted in the abdominal aorta via the femoral artery. The transmitter was placed subcutaneously. Depth of anesthesia was assessed as stability of respiratory movement and pupil size and paw-pinch reflexes. Mice were maintained on a 12:12-hour light-dark cycle. After the mice recovered from surgery (≥ 3 days), blood pressure and HR were sampled continuously for 10 seconds every 10 minutes. At day 7, MAP and HR were collected at 10-minute intervals 4 hours before and 3 days after LPS administration (20 mg/kg, intraperitoneally; *Escherichia coli*, serotype 0127:B8, L-3137, Sigma). The LPS dose was based on pilot studies of mortality in BK β1-KO and WT mice, where mortality was significantly greater in BK β1-KO compared to WT mice (Results). All animals were followed (including hemodynamic measurements) for up to 3 days.

Blood Sample and Tissue Collections

At 6 or 22 hours after LPS or saline administration, WT and BK β1-KO mice were euthanized with pentobarbital (80–100 mg/kg, intraperitoneally), and venous blood was collected and centrifuged to separate the serum, which was frozen at −80 °C. Tissues were collected at 22 hours after saline or LPS administration only. Left kidney, left lobes of the lung and liver, and small intestine segments were fixed in 4% paraformaldehyde for 24 hours before hematoxylin-eosin staining. Right kidney, inferior lobe of lung and median lobe of liver, and segments of small intestine were frozen at −80 °C until used for assay of myeloperoxidase activity (MPO) (See Methods, Supplemental Digital Content 1, http://links.lww.com/JCVP/A54). A branch of the mesenteric artery (MA) was isolated for contractile studies. MA and left ventricle were frozen at −80 until used for measurement of iNOS expression.

Measurement of Vascular Contractility in Vitro

MA (60 mmHg pressurized inner diameter ≈150–200 μm) were mounted in a pressure myograph with Krebs solution equilibrated with compressed air (5% CO_2-21% O_2-74% N_2, 37°C).[14]. Changes in inner diameter (ID) caused by paxilline (0.5 μM, a selective BK channel antagonist) and norepinephrine (NE, 0.1 nM – 1 μM) were recorded.

iNOS Expression in MA and Left Ventricular Myocardium

MA and left ventricles were homogenized and equivalent amounts of MA and myocardial protein (100 μg) from saline or LPS treated WT and BK β1-KO mice were separated on 7% SDS-polyacrylamide gels. The separated proteins were transferred to nitrocellulose membranes for western analyses using mouse anti-iNOS (1:2000; BD Transduction Laboratories, USA) antibody. β-actin (1:5000; Sigma) was used to verify equal protein loading. The iNOS positive control protein was obtained from lysates of mouse macrophages stimulated with interferon γ and LPS for 12 hours (BD Transduction Laboratories).

Morphological Assessment of Organ Damage and Polymorphonuclear Neutrophil Accumulation in Lung, Liver, Kidney and Small intestinal tissues

Organ damage was examined in hematoxylin-eosin-stained sections of lung, liver, kidney and small intestine (duodenum, jejunum, and ileum). The slides were coded, randomized, and assessed blindly. Damage scores were assessed as described (see Methods, Supplemental Digital Content 1, http://links.lww.com/JCVP/A54). Polymorphonuclear Neutrophil (PMN) accumulations were also examined in the sections of lung, liver and kidney using immunocytochemistry staining (see Methods, Supplemental Digital Content 1, http://links.lww.com/JCVP/A54). The assessment of PMN in tissues of duodenum failed due to the pealing of mucosa from the slices during the staining process

Assays for Tumor Necrosis Factor α and Interleukin 6 Production

Tumor Necrosis Factor (TNF) α and Interleukin 6 (IL-6) production were measured using ELISA with mouse TNF-α (BD Biosciences) and IL-6 kits (eBioscience, San Diego, CA).

Cecal Ligation/Puncture

Under isoflurane anesthesia (2–3%), the cecum was exposed through an abdominal midline incision. The cecum was ligated at positions that induce mid-grade sepsis and survival rates of 40% in WT mice.[19] Cecal puncture was performed using 24-gauge hypodermic needles and punctures were made in the mesenteric to antimesenteric direction (through-through). The abdominal incision was closed, and mice were returned to their cages and checked at 6-hour intervals for 7 days. To avoid the effects of analgesia on blood pressure, analgesics were not used after the induction of sepsis.

Statistics

Data are mean ± SE from n mice. Paired and unpaired *t* tests were used to two group comparisons. Multiple comparisons (concentration-response curves) were accomplished using a two-way ANOVA with repeated measures followed by Student-Newman-Keuls test.

Survival curves of WT and KO mice were fitted and compared by the Kaplan-Meier survival test. Data were analyzed using GraphPad Prism 4.0 software. *P* value <0.05 was considered statistically different.

Results

Dose Dependency of LPS-induced Mortality in BK β1-KO and WT Mice

To select a dose of LPS that allows us to differentiate between WT and BK β1-KO mice, we determined the dose dependency of LPS-induced mortality. At a dose of 10 mg/kg, LPS did not cause significant mortality in either group of mice (Fig 1). Significant mortality occurred at doses of 20 mg/kg and 40 mg/kg in both groups. At doses of 20 and 40 mg/kg, the latency to mortality was significantly shorter in BK β1 KO mice. Seventy-two hours post LPS, median survival time was 29 ± 7 hours (at 20 mg/kg) and 24 ± 6 hours (40 mg/kg) in endotoxemic BK β1-KO mice, significantly shorter than WT mice (43 ± 12 hours at 20 mg/kg and 40 ±16 hours at 40 mg/kg) (P<0.01 WT *vs* BK β1-KO) (Fig 1). At a dose of 80 mg/kg, mortality and median survival time was similar in WT (1/6) and BK β1-KO (0/6) mice (data not shown). Therefore, the dose of 20 mg/kg LPS was selected to examine LPS-induced hemodynamic changes and organ damage.

Exacerbated Hypotension, bradycardia and Mortality in Endotoxemic BK β1-KO Mice

The latency to first mortality was at 22 hour post-LPS in BK β1-KO mice; there were no survivors at 36 hours (Fig 2A). In WT mice, the latency to first mortality was 34 hours with 1 WT mouse surviving to day 7 (Fig 2A). Therefore, 22 hours post-LPS was chosen to investigate short-term consequences of BK channel function in endotoxemia.

MAP and HR from each group were averaged only up to 22 hours post-endotoxemia (Fig. 2B). Baseline MAP and HR were collected as 2-hour averages before LPS administration. LPS caused a tri-phasic response in all mice (Fig. 2B). The first phase was a transient fall of MAP in both groups of mice which was similar in WT and BK β1-KO mice (99 ± 3 to 62 ± 8 mmHg in WT mice, n=6; 103 ± 2 to 76 ± 7 mmHg in BK β1-KO mice, n=5). MAP initially recovered to control levels at 1 hour, and slowly fell again beginning 2 hours after LPS administration. The second fall in MAP in BK β1-KO mice was more rapid than in WT mice (−7.1 ± 1 *vs*. −4.3 ± 1 mmHg/h, P<0.05). The peak fall in MAP occurred 6 and 8 hours after LPS administration in BK β1-KO mice and in WT mice, respectively, but the peak drop in MAP was similar in BK β1-KO (62 ± 3 mmHg) and WT mice (67 ± 4 mmHg). Hypotension persisted in both groups until 22 hours, thereafter, BK β1-KO mice died (Fig. 2B). All mice had a transient elevation of MAP without increases in HR before they died (data was not shown).

LPS caused a rapid-onset bradycardia followed by a prolonged decrease in HR in both groups of mice (Fig. 2B). The transient bradycardia was similar in WT [540 ± 35 to 340 ± 50 beats per minute (bpm)] and BK β1-KO mice (520 ± 30 to 380 ± 70 bpm) (P>0.05). HR initially recovered to greater than pre-LPS levels by 3 hours but fell again in both groups of mice. The second fall in HR was similar in both groups of mice (685 ± 10 to 250 ± 5 bpm *vs* 710 ± 9 to 280 ± 50 bpm), but the fall in BK β1-KO mice was more rapid with the peak

decline occurring at 8 hours (−65 ± 2 bpm/h) (Fig. 2B), which didn't occur until 12 hours post-LPS in WT mice (−35 ± 6 bpm/h, P<0.05 vs BK β1-KO mice).

Impaired BK Channel Function and Reduced Reactivity to NE Without Elevation of iNOS Expression in MA at the Late Stage in Endotoxemic WT and BK β1-KO Mice

BK channel function and NE reactivity were tested *in vitro* in pressurized MA taken from mice 22 hours after saline or LPS treatment. In saline WT MA, paxilline (a BK channel blocker) constricted MA by 23 ± 6% (Fig 3A). Paxilline did not constrict LPS WT MA, saline-BK β1-KO MA or LPS-BK β1-KO MA (Fig 3A).

In saline-BK β1-KO MA, NE concentration response curves were left-shifted compared to WT MA (Fig 3B). pD2 values were 7.4 ± 0.4 for BK β1-KO MA and 6.5 ± 0.4 for WT MA (n=5–6, P<0.05). In LPS BK β1-KO and LPS WT MA, NE concentration-response curves were right-shifted compared to saline MA, and there was no difference in pD2 between the two groups.

iNOS expression did not differ in LPS WT and BK β1-KO MA compared to saline MA (Fig 3C). In myocardium, the basal level of iNOS was very low but expression increased in LPS BK β1-KO and WT mice.

Exacerbated Tissue Damage at the Late Stage in Endotoxemic BK β1-KO Mice

At 22 hours post-LPS administration, BK β1-KO lungs revealed more severe alveolar collapse, edema, inflammatory cell infiltration (Fig. 4C), and higher injury scores (Fig. 5A), compared to WT lungs (Fig 4B, 5A). BK β1-KO duodenum also exhibited greater loss of mucosal architecture (Fig. 4F) and higher injury scores (Fig. 5B), compared with WT duodenum (Fig. 4E, 5B). Similar data were obtained in the jejunum and ileum, although the injury was more severe in duodenum in both groups of mice.

BK β1-KO and WT livers displayed mild cytoplasmic vacuolation and focal nuclear pyknosis, leukocyte infiltration without necrosis (Fig. 4H, I), and similar injury scores (Fig. 5C). BK β1-KO and WT kidneys exhibited mild tubular cell swelling and tubular dilation, (Fig 4K, 4L), similar injury scores too (Fig 5D)

Elevated TNF-α and IL-6 levels in the Late, but not in the Early Phase in Endotoxemic BK β1-KO Mice

WT and BK β1-KO mice exhibited similar serum levels of TNF-α and IL-6 at 6 hours post-LPS administration. BK β1-KO mice had higher TNF-α and IL-6 levels than WT mice at 22 hours post LPS-administration. (Fig 6A, 6B).

Elevated Myeloperoxidase Activity and PMN Accumulation in Organs From Endotoxemia BK β1-KO Mice

At 22 hours post-LPS administration, BK β1-KO mice displayed higher Myeloperoxidase (MPO) activity and PMN accumulation in lung and liver, but not in kidneys (see Supplemental Data, http://links.lww.com/JCVP/A54).

Exacerbated Mortality in CLP-induced Septic BK β1-KO Mice

CLP-induced mortality was similar to LPS-induced mortality. The latency to mortality was 22 hours, and there were no survivors (0/13) at 50 hours post-CLP in non-resuscitated BK β1-KO mice (Fig 7).

Discussion

Previous studies suggested that activation of vascular BK channels contributes to the pathophysiology of septic shock by reducing vascular NE reactivity.[9,11–13] These studies indicate that a BK channel blocker should be beneficial in treating hypotension in shock. However, our data show that vascular SMC BK channel deficiency does not protect against hypotension in the early stage of endotoxemia. Furthermore, during the late stage of endotoxemia, BK β1-KO mice displayed more severe lung and intestinal injury than WT mice. Vascular BK channel deficiency shortened the latency to mortality and increased the mortality rate not only in LPS-induced sepsis, but also in CLP-induced sepsis. Control BK β1-KO MA had greater NE reactivity compared with WT MA. In late stage of endotoxemia, responses to NE and BK channel function were similarly impaired in BK β1-KO and WT MA without the increases in iNOS expression, which is thought to be responsible for vascular dysfunction in sepsis.[4] These data suggest that vascular BK channels have little or no role in LPS-induced hypocontractility to NE, but vascular BK channels may be crucial in maintaining organ function and survival in septic shock.

iNOS-derived NO activates BK channels,[21,22] which impairs NE-induced arterial constriction *in vitro*.[7,8] Early studies suggested that vascular BK channels may contribute to LPS-induced arterial hyporeactivity in endotoxemia *in vivo*,[9,10] and *in vitro*.[11–13] Therefore, blocking BK channels should protect against NO-induced vascular hyporeactivity to NE and hypotension in endotoxemia. Our data using BK β1-KO mice and recent studies in BK α-KO mice[14] indicate that the overall MAP responses to LPS and sepsis in WT and BK β1- or α-KO mice were similar.[14] In our mechanistic studies, we focused on the late stage (>12–16 hours post-LPS), and found: (1) endotoxemic BK β1-KO mice did not show higher *adrenergic vascular tone* compared with endotoxemic WT mice; (2) arterial BK channel function was impaired in endotoxemia, because paxilline-induced MA constriction was reduced in endotoxemic WT mice; (3) iNOS expression was not elevated in MA from both endotoxemic WT and BK β1-KO mice. These results do not support the ideal that vascular BK channels are activated and this contributes to hypotension during endotoxemia. Vascular BK channel function can be inhibited by the acidosis, hypoxia and oxidative stress that are known to occur in endotoxemia.[23,24] These additional complications of endotoxemia could account for impaired BK channel function in WT MA. It is still unclear why NE reactivity also was diminished in endotoxemic BK β1-MA in the absence of increased vascular iNOS expression as the role of iNOS-NO in septic shock is well established.[4] We speculate that elevated vascular iNOS expression in the early stage was declined in the later stage of endotoxemia in our studies, and we did detect an increase in myocardial iNOS expression in late stage endotoxemia. Our data are supported by the recent studies in BK α-KO mice.[14] In septic BK α-subunit KO mice, any activation of vascular BK channel should be blunted,

including iNOS-derived NO. Therefore, studies from both BK α- and β1-KO mice do not support that BK channel activation contributes to hypotension in septic shock.

Maintaining organ perfusion with volume resuscitation and/or maintenance of cardiac output is more important than controlling MAP in prevention of organ damage and death during septic shock.[2,25,26] Although hypotension and bradycardia occurred in LPS-treated mice in our studies, all mice showed a transient *increase* in MAP but not HR before they died. The transient increases in MAP might be due to elevated peripheral resistance which would further reduce perfusion of ischemia-sensitive organs via *non-adrenergic* vascular tone, possibly via enhanced Ca^{2+} channel activity since blocking L-type Ca^{2+} channels improves survival in septic shock.[27–30] Dysregulated L-type Ca^{2+} channel function would be more predominant in BK β1-KO mice because of the absence of feedback regulation by BK channels.[18] This suggestion is consistent with data from LPS-treated BK β1-KO mice which displayed more severe lung and intestinal damage, and higher mortality with a more rapid onset than WT mice. Higher mortality was also seen in septic BK α-subunit KO mice, but unfortunately, organ damage scores were not reported.[14] In our studies, we found that exaggerated organ damage did not occur uniformly across all tissues from endotoxemic BK β1-KO mice. Damage was more severe in the lung and small intestines, suggesting that BK channels may play a more prominent role in regulating organ perfusion in these tissues compared with the kidneys for example.[23,31,32] Therefore, future studies need to focus on measurements of organ blood flow, lactate/pyruvate or ketone body ratio, and tissue levels of ATP[33] in septic WT and BK β1-KO mice.

Although BK channel α-subunits are expressed in immune cells, BK β1-subunits are not. The role of BK channel in immune system function is unclear. BK channels were reported to be required for LPS-induced macrophage cytokine release[34], but more recent studies argue against this function.[35,36] Both septic BK α- and endotoxemic BK β1-KO mice (early stage) displayed exaggerated mortality and prolonged hypotension with similar cytokine levels compared with septic WT mice.[14] But organ injury and inflammatory responses were not uniform across all organs as more severe injury occurred in the ischemia sensitive small intestine in endotoxemic BK β1-KO mice. These data argue against a broad contribution of the immune system to organ damage and mortality. Instead, vascular dysfunction may be the key factor, because our use of SMC-specific BK β1-KO mice separates vascular from other systemic responses that may be involved in organ damage and hemodynamic regulation in septic shock. The higher levels of cytokines in the late stage of endotoxemic BK β1-KO mice may be caused by diminished clearance rather than enhanced release, as cytokine levels were higher in LPS-treated mice compared with polymicrobial septic mice. This needs to be confirmed by further detailed studies. In endotoxemic BK β1-KO mice, the pattern of organ inflammation was consistent with the organ damage. These results may imply that organ hypoxia and ischemia cause higher cytokine levels and enhanced organ inflammation in BK β1-KO mice at the late stage of endotoxemia.

Conclusions

BK β1-KO mice are more sensitive than WT mice to LPS- or CLP-induced septic shock, as reflected by more severe organ damage, shorter latencies to death and higher mortality rates.

Although arteries from normal BK β1-KO mice exhibit increased reactivity to NE *in vitro*, these mice do not have higher systemic blood pressures before or during endotoxemia. In the later stages of endotoxemia, arterial BK channel function is inhibited in WT mice. BK channel activation may have a beneficial role in protecting against organ damage and mortality in septic shock, but the mechanisms are unclear. Although higher mortality was shown in both endotoxemic and CLP-BK β1-KO mice, further mechanistic studies need to be done in CLP-septic model, a more realistic and clinically relevant model for the induction of polymicrobial sepsis.

Perspective

Septic shock is associated with high morbidity and mortality but few effective treatments are available.[2,26] Sepsis and sepsis-induced mortality are more common in elderly and diabetic patients who have altered vascular tone and impaired vascular BK channel functions, suggesting that there may be interactions between BK channel function and septic shock.[37–40] Our studies provide new information about the causes of hemodynamic dysfunction in septic shock. These studies suggest a potential new target for therapeutic intervention in septic shock: vascular BK channel activators. Tissue hypoperfusion is an important factor in the development of multiple organ failure. Understanding these hemodynamic abnormalities and cellular mechanisms responsible for tissue hypoperfusion will help develop treatments that will reduce organ injury and mortality in septic shock.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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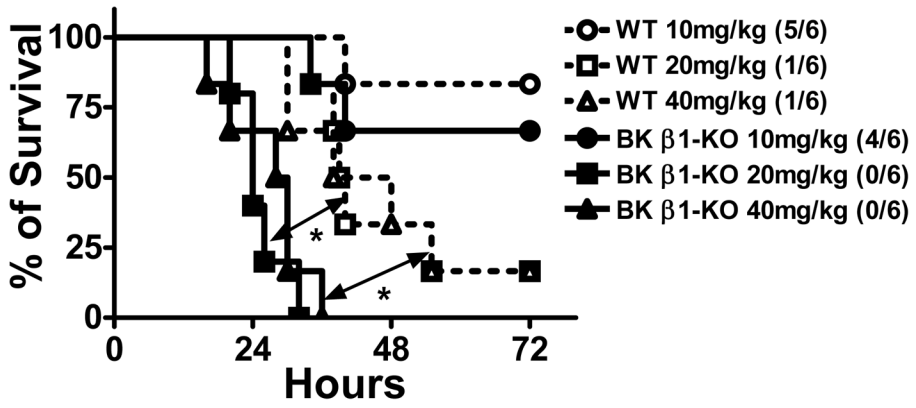

Figure 1.

Dose dependency of mortality in endotoxemic WT and BK β1-KO mice. LPS was used at 10, 20 and 40 mg/kg (intraperiotoneal administration), and survivals were counted for 3 days for each group. BK β1-KO mice are more sensitive to LPS-induced the mortality. * Significantly different from WT mice ($P<0.05$)

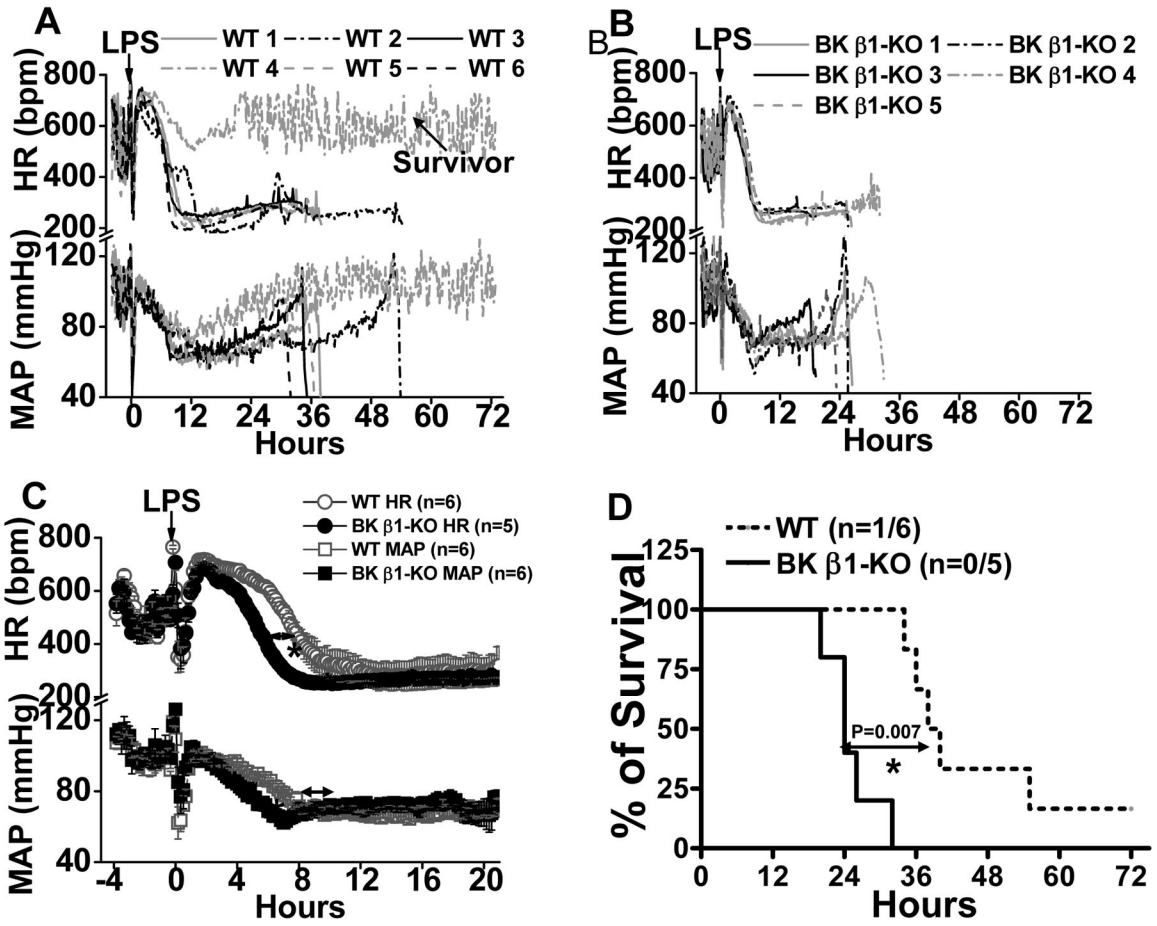

Figure 2.

A, Three day survival rate in WT and BK β1-KO mice. The latency to mortality was shorter in BK β1-KO mice. B, Continuous measurement of MAP in telemetry-implanted WT and BK β1-KO mice after LPS (20 mg/kg, intraperiotoneally). MAP and HR were sampled for 10 seconds every 10 minutes. MAP and HR from WT and BK β1-KO mice were averaged before and 22 hours after LPS administration; the parameters cannot be averaged after this time point because of decreased numbers of BK β1-KO mice. * Significantly different from WT mice ($P<0.05$)

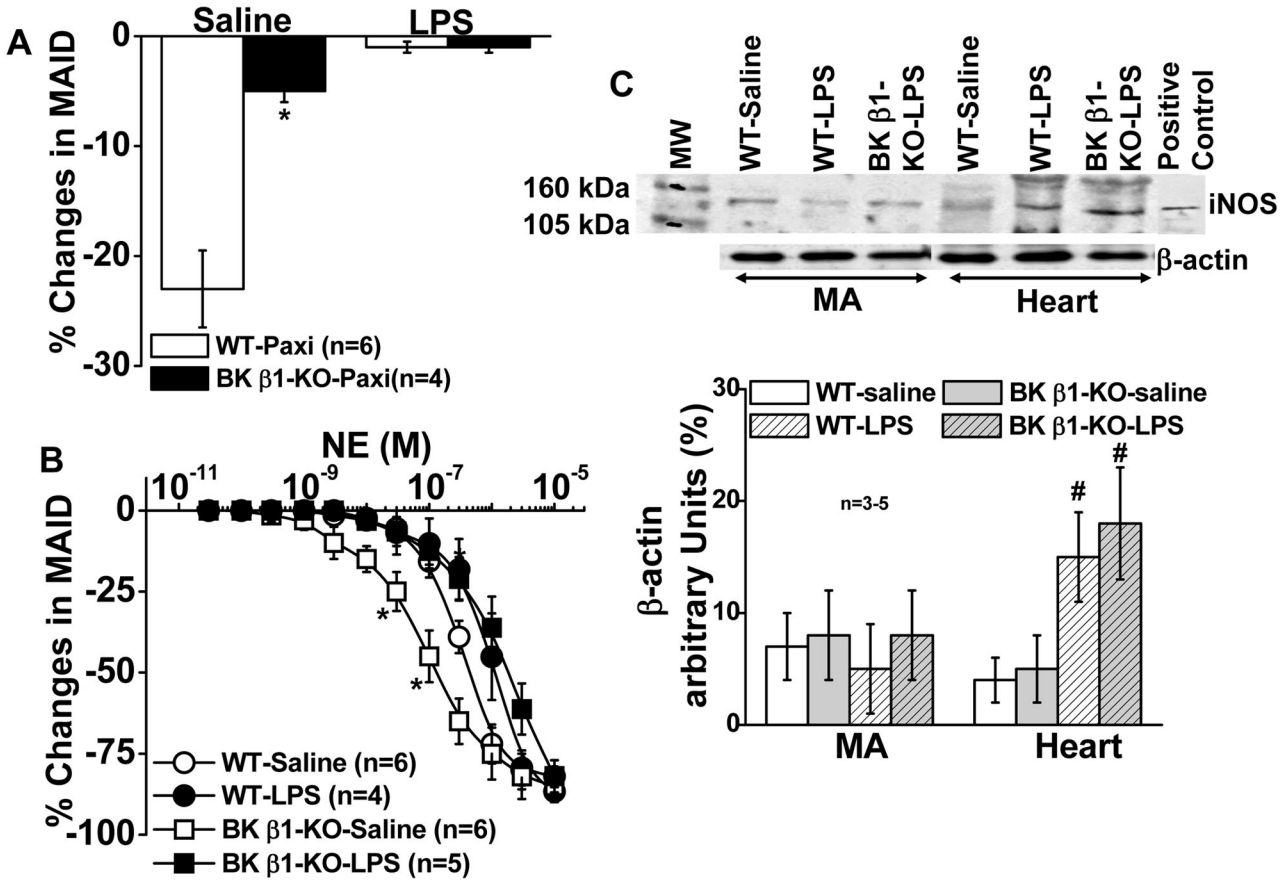

Figure 3.

Effects of paxilline (Paxi) and NE on MA 22 hours after saline or LPS treatment of WT and BK β1-KO mice. A, Contractile responses to Paxi (0.5 μM) in saline and LPS WT and BK β1-KO MA. B, Contractile responses to NE in saline or LPS WT and BK β1-KO MA. C, Expression of iNOS in MA and myocardial lysates 22 hours after saline or LPS treatment. Lane 1 is molecular weight, lane 2–7, the samples of MA and myocardium from individual mice, lane 8, the positive control protein. * Significantly different from WT MA ($P<0.05$)

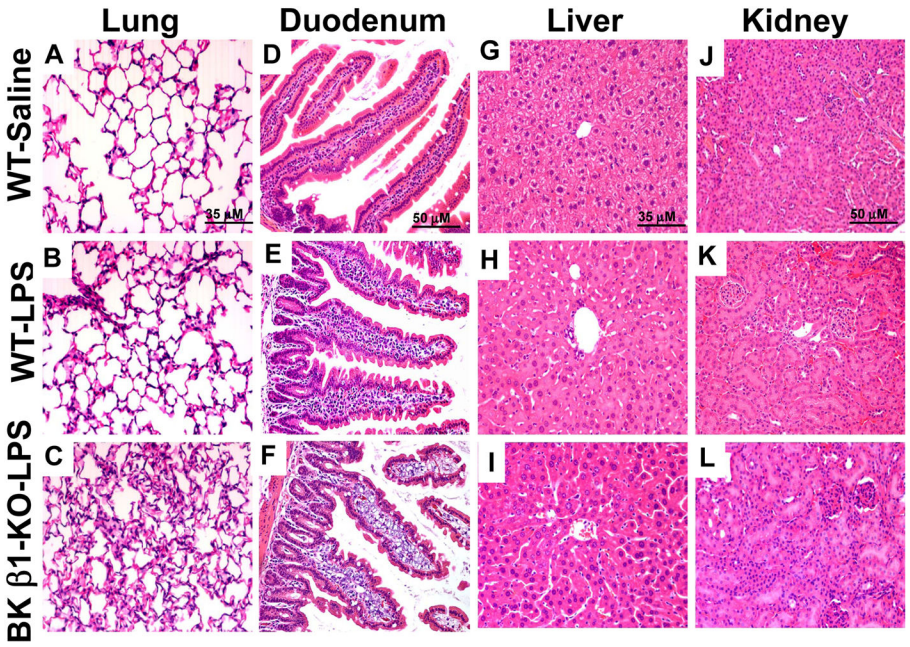

Figure 4.

Hematoxylin-eosin-stained slices of lung (A–C), duodenum (D–F), liver (G–I) and kidney (J–L) tissues from 22 hours after saline or LPS treated WT and BK β1-KO mice.

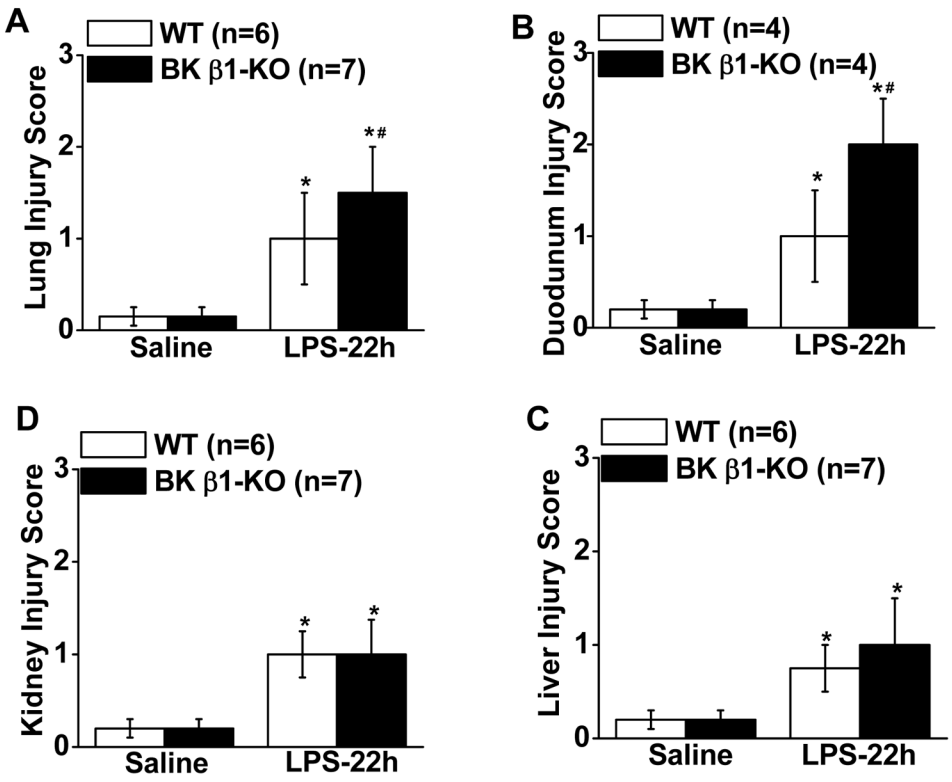

Figure 5.

Comparison of tissue injury score in lung (A), duodenum (B), liver (C), and kidney (D) from 22 hours after saline or LPS-treated WT and BK β1-KO mice. * Significantly different from saline treated WT mice. # Significantly different from LPS treated WT mice. ($P<0.05$)

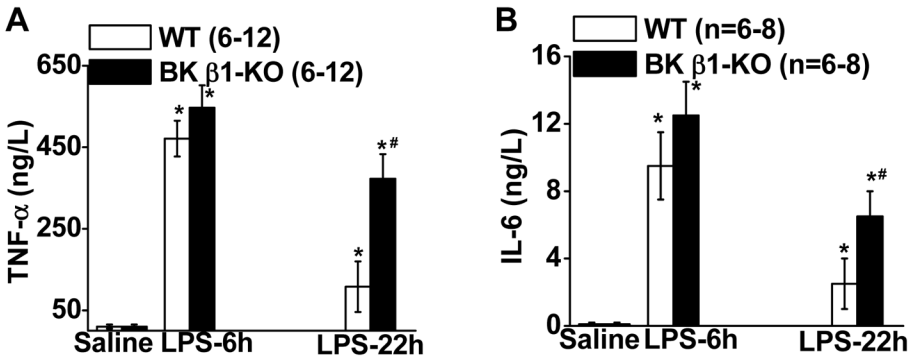

Figure 6.

Comparison of plasma TNF-α and IL-6 levels in untreated, and 6 or 22 hours post-LPS treated WT and BK β1-KO mice. * Significantly different from untreated mice. # Significantly different from LPS treated WT mice. ($P<0.05$)

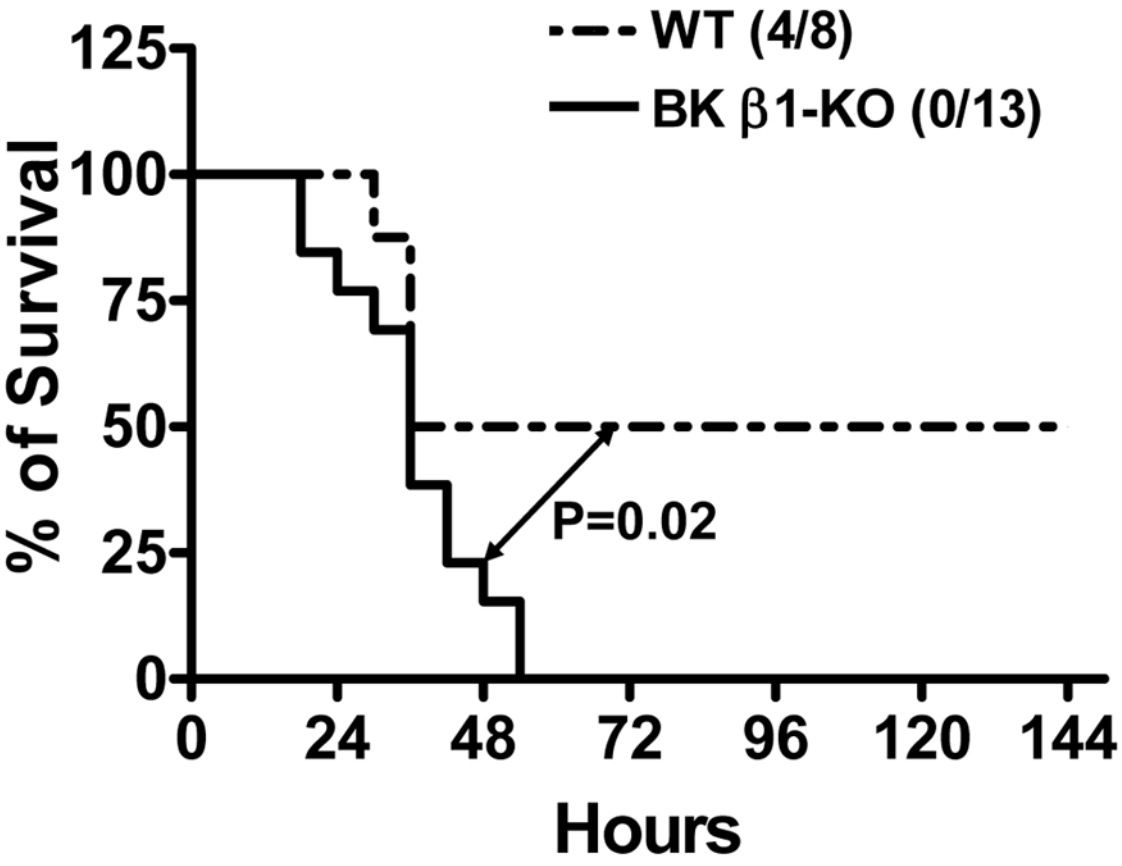

Figure 7.

Comparison of 7-day survival rate in CLP-induced septic shock in WT and BK β1-KO mice. The survival rate was significantly low in BK β1-KO mice. * Significantly different from WT mice. ($P<0.05$)