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Research Article

BK_{Ca} channels are involved in spontaneous and lipopolysaccharide-stimulated uterine contraction in late gestation mice†

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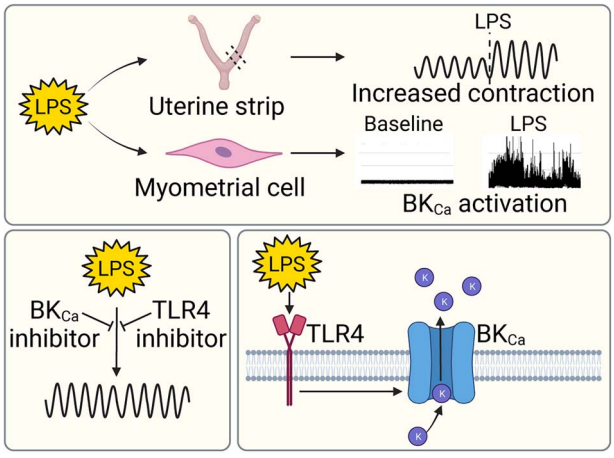
Abstract

The large-conductance, voltage-gated, calcium (Ca^{2+})-activated potassium channel (BK_{Ca}) is one of the most abundant potassium channels in the myometrium. Previous work conducted by our group has identified a link between inflammation, BK_{Ca} channels and excitability of myometrial smooth muscle cells. Here, we investigate the role of BK_{Ca} channels in spontaneous and lipopolysaccharide (LPS)-stimulated uterine contraction to gain a better understanding of the relationship between the BK_{Ca} channel and uterine contraction in basal and inflammatory states. Uteri of C57BL/6 J mice on gestational day 18.5 (GD18.5) were obtained and either fixed in formalin or used immediately for tension recording or isolation of primary myocytes for patch-clamp. Paraffin sections were used for immunofluorescenctdetection of BK_{Ca} and Toll-like receptor (TLR4). For tension recordings, LPS was administered to determine its effect on uterine contractions. Paxilline, a BK_{Ca} inhibitor, was used to dissect the role of BK_{Ca} in uterine contraction in basal and inflammatory states. Finally, patch-clamp recordings were performed to investigate the relationship between LPS, the BK_{Ca} channel and membrane currents in mouse myometrial smooth muscle cells (mMSMCs). We confirmed the expression of BK_{Ca} and TLR4 in the myometrium of GD18.5 mice and found that inhibiting BK_{Ca} channels with paxilline suppressed both spontaneous and LPS-stimulated uterine contractions. Furthermore, application of BK_{Ca} inhibitors (paxilline or iberiotoxin) after LPS inhibited BK_{Ca} channel activity in mMSMCs. Moreover, pretreatment with BK_{Ca} inhibitor or the TLR4 inhibitor suppressed LPS-activated BK_{Ca} currents. Our study demonstrates that BK_{Ca} channels are involved in both basal and LPS-stimulated uterine contraction in pregnant mice.

Summary Sentence
LPS stimulates uterine contraction in part, by activating BK_{Ca} membrane currents in mMSMCs.

Graphical Abstract



Key words: BKCa channel, LPS, myometrium, contraction

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Introduction

During pregnancy, the uterus is relatively quiescent, producing asynchronous and regional contractions that permit the growth of fetus. At term, the uterus becomes more sensitive to contractile stimuli, and synchronized phasic contractions develop and increase to facilitate labor [1]. Excessive contractility endangers both fetus and mother by increasing the risk for fetal hypoxia and uterine rupture [2, 3]. Conversely, insufficient contractility may increase the risk for both cesarean delivery and morbidity and mortality for the mother and fetus [4, 5]. Thus, better understanding of the mechanisms controlling the uterine transition from quiescence to contractile is essential to ensure the health of mothers and newborns.

Uterine contraction arises, in part, due to changes in electrical activity of the smooth muscle cells of the myometrium [6, 7]. Two ions, key to regulating of the electrical activity of myometrial smooth muscle cells (MSMCs) are calcium (Ca^{2+}) and potassium (K^+). Ca^{2+} influx through Ca^{2+} channels depolarizes the myometrial smooth muscle membrane and activates the contractile machinery, while K^+ efflux through K^+ channels repolarizes the membrane and returns the cell to the resting state [8, 9]. The large-conductance, voltage-gated, calcium (Ca^{2+})-activated potassium channel (Kcnma1), also known as BK_{Ca}/MaxiK /KCa1.1/hSlo, is one of the most abundant potassium channels in the myometrium [10–13] and is implicated in maintaining the quiescence of uterine smooth muscle [14]. Studies have demonstrated that blocking BK_{Ca} channels depolarized MSMCs and increased myometrial contractility [15], while activation of BK_{Ca} channels evoked a large efflux of K^+ and repolarization of membrane [16]. Other studies showed a minimal effect of BK_{Ca} channel openers or blockers on myometrial contraction in vitro [17, 18]. Due to these conflicting findings, the role of this channel in regulating uterine contractions remains controversial.

Inflammation is known to induce uterine activation and parturition [19–21]. Lipopolysaccharide (LPS) is a pathogen-associated molecular pattern molecule from Gram-negative bacteria, which usually binds Toll-like receptor 4 (TLR4) to trigger a proinflammatory cascade [22]. Previously, we reported that LPS could induce contraction in primary and immortalized human MSMC (hMSMC) cells using a collagen-gel–based contraction assay [23]. We also found that the anti-inflammatory factor, activated alpha-2-macroglobulin, regulates BK_{Ca} channel through modulating myometrial $[Ca^{2+}]_i$ dynamics in human MSMCs [24]. These findings indicate a strong relationship between inflammation, BK_{Ca} channels, and uterine contraction, the mechanism of which is unclear.

In the current study, we investigate the role of BK_{Ca} channels in uterine contraction and MSMC electrical activity in both the basal state and after an inflammatory stimuli. To understand the role of this channel in late pregnancy, we blocked BK_{Ca} channel activity with BK_{Ca} inhibitors in uterine strips and primary mouse MSMCs (mMSMCs) from C57BL/6 J mice on gestational day 18.5 (GD 18.5) in the presence and absence of LPS. Uterine contraction was measured with tension recordings and electrical activity in mouse MSMCs was evaluated by patch-clamp recordings. We aim to clarify the role of the BK_{Ca} channel in uterine contraction of late gestation mice and lead to better understanding of the relationships between inflammation, BK_{Ca} channels, and uterine contractile activity.

Materials and methods

Animals and tissue collection

All animal work complied with the Guidelines for the Care and Use of Laboratory Animals set forth by the NIH and protocols approved by the Animal Care and Use Committee at Washington University in St Louis School of Medicine. C57BL/6 J mice (3–6 months old; Jackson Laboratories, Bar Harbor, ME) were housed under controlled laboratory conditions with a 12/12 h light–dark cycle for at least 1 week. Females were paired with a male for 2 h; those that had a copulatory plug were considered pregnant and were designated as gestation day (GD) 0 of pregnancy (GD 0). Mouse uterine samples were collected from pregnant mice on GD 18.5 after 5 min CO_2 euthanasia and were fixed in 10% formalin for 48 h or immediately used for tension recording or isolation of primary myocytes.

Immunofluorescence microscopy

Fixed uteri were processed and embedded in paraffin using a standard protocol. All sections (4 μm) were deparaffinized with xylene and hydrated through graded ethanol. Antigen retrieval was performed with 10-mM Sodium Citrate Buffer (pH 6.0) on medium power in a microwave for 15 min in 3×5 min intervals. Nonspecific staining was blocked with 10% normal goat serum for 30 min at 37°C; then, primary antibodies, secondary antibody-HRP conjugates, and TSA Dyes were applied and incubated on slides sequentially according to the instruction of goat anti-mouse/rabbit multiplex IFC detection kit (Catalog#18003, Zenbio, Chengdu, China). The primary antibodies used were rabbit anti-BK_{Ca} (Catalog # APC-151, Alomone, Israel, 1:200), mouse anti-TLR4 (Catalog # MA516216, Thermo Fisher Scientific, Waltham, MA, USA, 1:200), and Rabbit anti-smooth muscle actin (Catalog # ab5694, Abcam, Cambridge, UK, 1:4000). Simultaneously, the negative control antigen (blocking peptide for BK_{Ca}) was preincubated with Rabbit anti-BK_{Ca} antibody at room temperature for 30 min and was used as a negative control; the antibody diluent (PBS with 0.3% Triton™ X-100) was used as another negative control (Supplementary Figure S1). The steps of antigen retrieval to dye incubation were repeated three times until the various tyramine fluorescein substrates were incubated, then nuclei were visualized using DAPI staining (Vector, Burlingame, CA, USA). All images were acquired using a Leica DMi8 fluorescence microscope (Leica, Wetzlar, Germany) with Leica Application Suite X software.

Tension recording

Tissue from the cervical end of the uterus was dissected in cold Krebs solution (133-mM NaCl, 4.7-mM KCl, 1.2-mM $MgSO4.7H_2O$, 1.2-mM KH_2PO_4, 1.2-mM $CaCl_2.2H_2O$, 10-mM TES, and 11.1-mM glucose, PH7.4) to obtain 4 mm by 8 mm strips of longitudinal muscle, excluding the mesometrial border. Strips were mounted to force transducers in organ baths filled with oxygenated Krebs solution (95% O2, 5% CO2) at 37°C, and tension was recorded with a Powerlab data acquisition system (AD Instruments, Castle Hill, NSW, Australia). Basal tension (1.5 g) was gradually applied to strips, and tissue was equilibrated for 40–50 min prior to study. Following the equilibration period, spontaneous contractions were recorded for 15 min to establish a baseline, followed by either treatment with LPS (20 μg/ml, Catalog # L2630,

from *Escherichia coli* O111: B4, Sigma-Aldrich) or paxilline (40 μM, Catalog # 57186-25-1, Tocris Bioscience). The doses of LPS and paxilline were determined based on previous studies [23, 25] or dose trials (Supplementary Figure S2). The time was recorded from the first treatment as 0 min. At the end of experiment, 50-mM KCl was added to confirm tissue viability. Timed and vehicle control (DMSO for paxilline and water for LPS) experiments were performed simultaneously. LabChart 8 software (ADInstruments, Bella Vista, Australia) was used to analyze the tension recording data. The dose response module was chosen to calculate integral (Area under curves, AUC) and average amplitude with per-dose baseline correction. Frequency was counted every 15 min. All the values were normalized to the baseline of the same channel 15 min prior to the first treatment [26].

Isolation and primary culture of MSMCs

Uteri were removed and placed in sterile ice-cold $Ca^{2+}$and Mg^{2+} free DPBS (Fisher Scientific, Hampton, NH), dissected longitudinally, cleaned of mesentery, fetal tissue, and membranes, and carefully scraped to remove the endometrium. The myometrium was then washed in DPBS twice and chopped into smaller pieces. Myometrial pieces were digested using liberase TM (0.25 U/ml; Catalog # 05401119001, Sigma) diluted in DPBS at 37°C with rotation for 45–50 min. During the incubation, tissue pieces were pipetted up and down several times. Following the incubation, an equal volume of DMEM with 10% FBS was added to the suspension, and the solution was filtered through 100-μM cell strainer. Filtered cells were centrifuged at 400 g for 5 min at room temperature, pelleted, resuspended in 10% FBS DMEM, and plated with smooth muscle growth supplement (SMGS, Catalog #S00725, Gibco). For all experiments, mMSMCs were used at passage 1 or 2 to ensure the purity of the cells. Alpha-smooth muscle actin (SMA) and h-caldesmon were used as molecular markers to identify MSMCs (Supplementary Figure S3).

Electrophysiology

Patch pipettes were pulled from borosilicate glass capillaries with a resistance of 3–8 MΩ. The electrodes were filled with a solution containing (mM): 140 KCl, 10 EGTA, 3 MgCl2, 10 Hepes, and 10 glucose (pH 7.4). Cell-attached patch-clamp recordings were performed on mouse MSMCs at room temperature in a bath solution containing (mM):140 KCl, 20 NaCl, 2.4 CaCl2, 6.1 MgCl2,10 Hepes (pH 7.4) with a holding potential of −60 mV. Recordings were acquired at 100 kHz and filtered at 5 kHz. The single-channel open-state probability (*Po*) was calculated using pCLAMP software. Single-channel currents were recorded for at least 5 min before adding any reagents or vehicle to ensure that the membrane current was stable. For these patch-clamp experiments, LPS (10 ng/ml, Catalog# L2630, from *E. coli* O111,B4, Sigma-Aldrich), Paxilline (20 μM, Catalog # 57186–25-1, Tocris Bioscience), Iberiotoxin (10 nM, Catalog #120379, Abcam), and TAK-242 (1 μM, Catalog # 508336, Sigma-Aldrich) were used. Cell Counting Kit-8 (Catalog # SX538, Dojindo, Japan) was used to assess the 3-h effects of these agents on cell viability (Supplementary Figure S4).

Statistical analysis

Data are presented as mean ± SEM. Statistical analyses were performed by Student's *t* test (two groups) or one-way ANOVA (three or more groups) with post-hoc test (Bonferroni), using GraphPad Prism 8 (San Diego, CA, USA) software and SPSS 20 software (IBM, Armonk, NY). A *P*-value <0.05 was considered significant.

Results

BK_{Ca} and TLR4 are expressed in mouse myometrium

The expression of the BK_{Ca} subunit (alpha) in the uterus of C57BL/6 J mice at GD 18.5 was investigated using immunofluorescence methods. The BK_{Ca} channel and TLR4 are expressed not only in endometrium as reported [27, 28] but also in myometrium as show by co-expression with smooth muscle actin (SMA) (Figure 1) [29]. In addition to the myometrium, a few cells in the endometrium express SMA, which likely represents vascular endothelial cells based on their circular distribution and morphology. The co-expression of the BK_{Ca} channel and TLR4 in the mouse myometrium at GD 18.5 suggests that they may have a role in regulating the function of the myometrium.

LPS stimulates uterine contraction of pregnant mouse in vitro

To investigate the role of BK_{Ca} channels in regulating uterine contraction in an inflammatory state, we first established an ex-vivo LPS-stimulated uterine contraction model by measuring tension in uterine strips isolated from GD 18.5 mice. LPS (20 μg/ml) gradually increased the amplitude of uterine contractions (Figure 2A), with an average increase of 137.43% of baseline at 45–60 min ($P < 0.05$) (Table 1). AUC, which reflects the combination of amplitude, contraction

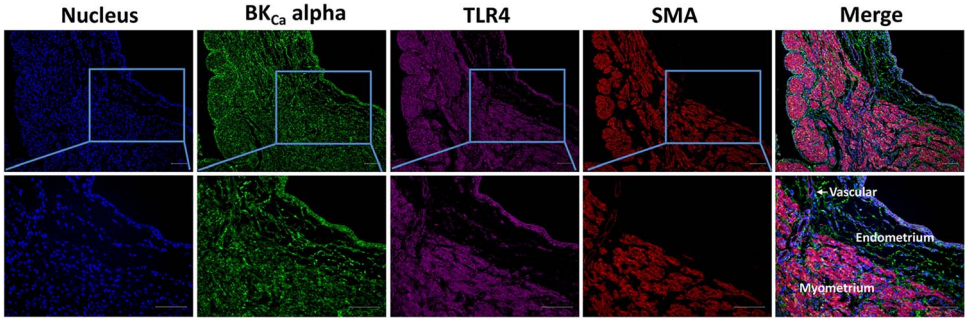

Figure 1. Immunofluorescence staining of BK_{Ca} alpha and SMA in uterine tissues from C57BL/6 *J mouse at GD 18.5.* Representative images show the localization of BK_{Ca} alpha, Toll-like receptor 4 (TLR4), and smooth muscle actin (SMA) in gestational day 18.5 (GD 18.5) in mouse uterus. SMA marks smooth muscle cells. Nuclei were visualized with DAPI. Scale bar = 100 μm.

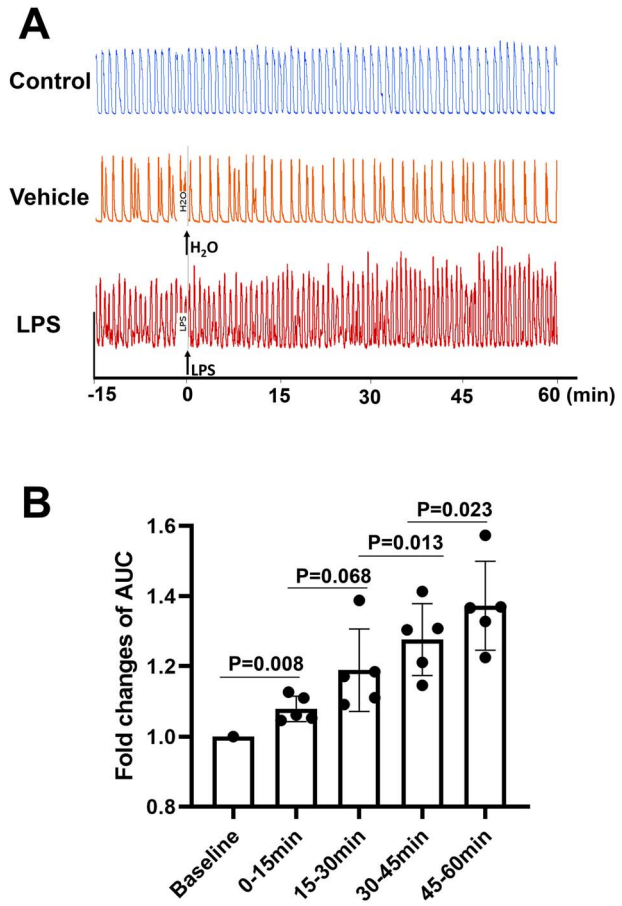

Figure 2. LPS stimulated uterine contractions of pregnant mice in vitro. (A) Representative tension recordings of time control, vehicle control (water), and LPS (20 μg/ml) treated GD 18.5 uterine strips. Arrowheads indicated the application of LPS or vehicle. (B) Fold changes in AUC of LPS-treated uterine strips were calculated and normalized according to the baseline every 15 min. Data are presented as mean ± SEM from five independent experiments.

duration, and frequency, also increased over time and significantly increased immediately (0–15 min) after LPS treatment ($P < 0.05$) (Table 1; Figure 2A and B). Since we did not detect differences in the frequency of contractions in the presence of LPS ($P > 0.05$), the increases in AUC reflect changes in amplitude (Figure 2B).

Timed and vehicle control experiments were performed simultaneously, and the results showed no significant changes in amplitude, frequency, and AUC during measuring time.

Inhibiting BK_{Ca} channels with paxilline decreases both spontaneous and LPS-stimulated uterine contraction

To test if BK_{Ca} channels are involved in regulation of both spontaneous and inflammatory-induced uterine contractions, uterine strips were treated with LPS (20 μg/ml) and/or BK_{Ca} inhibitor paxilline (40 μM) (Figure 3A). Paxilline gradually decreased AUC of spontaneous uterine contractions ($P < 0.05$), which resulted from a decrease in amplitude ($P < 0.05$). When paxilline was applied 15 min after LPS, the stimulatory effect of LPS was inhibited and AUC was decreased ($P < 0.05$). Likewise, uterine contractions that initially inhibited by paxilline ($P < 0.05$) were not recovered by the addition of LPS (Figure 3A and B and Table 1). The AUC from the final 15 min of the recording period was compared with baseline to show cumulative changes in contraction resulting from LPS and/or paxilline treatments (Figure 3C). AUC was continually dampened for up to 1 h when BK_{Ca} channels were inhibited indicating that LPS is regulating uterine contraction, in part, by acting through BK_{Ca} currents.

Vehicle control (DMSO for paxilline) experiments were also recorded simultaneously and no significant differences were found from baseline (Supplementary Figure S5).

LPS activates BK_{Ca} membrane currents in mMSMCs

To investigate the possible role of BK_{Ca} channels in response to LPS, we used the cell-attached patch-clamp recording configuration to leave the cytoplasmic content intact and recorded at a holding potential of −60 mV in mMSMCs. We observed that the BK_{Ca} channel currents in the unstimulated states had a very low open-state probability (P_o), which was 0.0003 ± 0.0001. In the presence of LPS (10 ng/ml), BK_{Ca} currents increased immediately and dramatically, and the P_o increased significantly to 0.1364 ± 0.0277 (Baseline vs LPS, $P < 0.001$). The subsequent application of paxilline (20 μM) or iberiotoxin (10 nM) suppressed most of the LPS-induced membrane currents (Figure 4A–B), and the P_o decreased to 0.000879 ± 0.000398 and 0.0031 ± 0.0022, respectively. (LPS vs LPS + Pax, $P < 0.001$; LPS vs LPS + IBTX, $P < 0.001$)

Table 1. Fold changes of contraction in different groups.

Group	Parameter	Baseline	0–15 min	15–30 min	30–45 min	45–60 min	*P*-Value
LPS	AUC (%)	100.00 ± 0.00	107.89 ± 1.63	118.89 ± 5.27	127.61 ± 4.57	137.22 ± 5.66	0.001
	Amplitude (%)		107.89 ± 1.63	118.91 ± 5.27	127.05 ± 4.80	137.43 ± 5.60	0.001
	Frequency (%)		105.52 ± 3.39	99.84 ± 2.90	97.65 ± 3.16	94.82 ± 2.48	0.132
Paxilline	AUC (%)	100.00 ± 0.00	70.61 ± 12.61	47.45 ± 17.85	39.37 ± 14.56	34.46 ± 12.56	0.023
	Amplitude (%)		71.09 ± 12.29	49.21 ± 16.73	39.38 ± 14.56	34.55 ± 12.50	0.023
	Frequency (%)		98.81 ± 2.83	95.45 ± 4.55	89.02 ± 4.29	85.75 ± 6.56	0.145
LPS + Pax	AUC (%)	100.00 ± 0.00	105.18 ± 0.87	83.87 ± 5.42	61.92 ± 6.64	51.50 ± 5.12	0.001
	Amplitude (%)		105.19 ± 0.86	83.88 ± 5.43	61.92 ± 6.65	51.50 ± 5.12	0.001
	Frequency (%)		97.78 ± 1.36	101.67 ± 5.53	102.94 ± 6.94	99.96 ± 7.48	0.688
Pax+LPS	AUC (%)	100.00 ± 0.00	82.22 ± 2.97	67.62 ± 6.13	53.43 ± 5.27	43.96 ± 8.27	0.001
	Amplitude (%)		82.18 ± 2.96	67.59 ± 6.10	53.41 ± 5.27	43.97 ± 8.26	0.001
	Frequency (%)		99.82 ± 7.69	110.08 ± 6.68	102.86 ± 15.76	107.70 ± 20.42	0.663

Data are presented as mean ± SEM from 4 to 5 independent experiments. The *P*-value was obtained by one-way ANOVA test. Pax, paxilline.

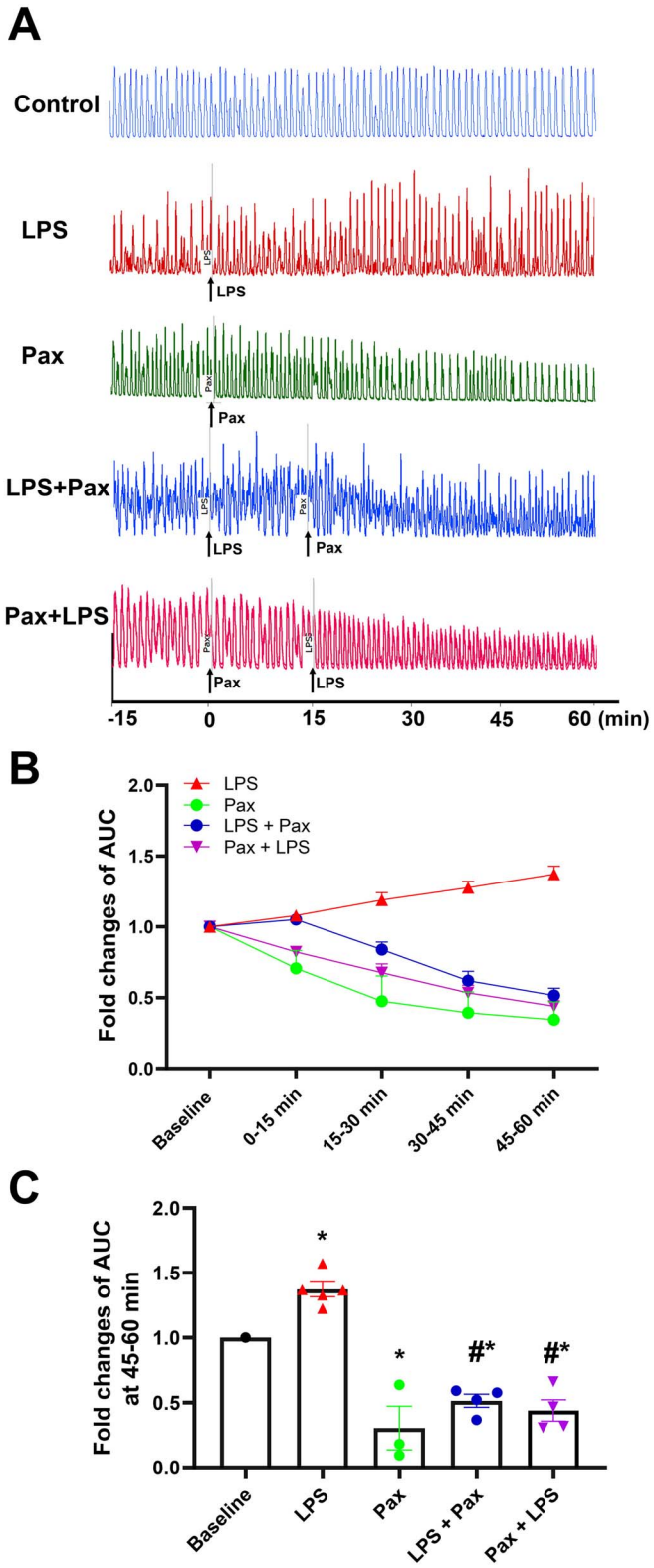

Figure 3. Paxilline decreased both spontaneous and LPS-stimulated uterine contractions. (A) Representative tension recordings, including time control (Control), Pax (40 μM), LPS (20 μg/ml), LPS + Pax, and Pax + LPS. Arrowheads indicated the application of LPS or Paxilline. (B) Fold changes in AUC of paxilline alone, LPS, LPS + Pax, and Pax + LPS groups were calculated and normalized according to the baseline every 15 min. (C) Fold changes in AUC from the final 15 min of the recording period (at 45–60 min) were normalized and compared with baseline or LPS group to show cumulative changes in contraction resulting from LPS and/or paxilline treatments. Pax, paxilline. Data are presented as mean ± SEM from 4 to 5 independent experiments. *, $P < 0.050$, compared with baseline; #, $P < 0.050$, compared with LPS.

(Figure 4C). These data indicated that LPS acts through BK_{Ca} membrane currents.

To further confirm the possible role of BK_{Ca} channels involved in LPS effects, paxilline (20 μM) or iberiotoxin (10 nM) were applied to inhibit the BK_{Ca} channels 10 min prior to the application of LPS. The membrane currents and P_o did not increase as seen with LPS alone (0.0002 ± 0.0001 vs 0.0003 ± 0.0002 vs 0.0002 ± 0.0001, baseline vs Pax + LPS vs IBTX + LPS, $P > 0.05$) (Figure 4D and E). Namely, pretreatment with BK_{Ca} inhibitor suppresses LPS-activated membrane currents.

TLR4 mediates the activation of membrane currents induced by LPS

TLR4 is the primary receptor for LPS in MSMCs and can be blocked with TAK-242 [30]. To determine if TLR4 was responsible for the increased currents in mMSMCs following LPS treatment, we blocked TLR4 with TAK-242 (1 μM) before the application of LPS. We found that LPS failed to increase P_o after pretreatment with TAK-242 (Figure 5A and B), indicating that LPS-activation of BK_{Ca} currents occurs through TLR4 signaling.

Discussion

Uterine contractility is delicately regulated during pregnancy to ensure an appropriate length of gestation and successful parturition. Inflammation is widely accepted as one of the initiators in uterine contraction [31], while the intensity of uterine contractility primarily depends on the excitability of MSMCs, which is modulated by multiple ion channels [8, 32]. However, little is known about the interactions between inflammation, ion channels, and uterine contraction [33], including the contribution of the most predominant ion channels in MSMCs, the BK_{Ca} channel [14–16, 18]. In this study, we demonstrate that LPS exposure causes an immediate and significant increase of BK_{Ca} membrane currents in mMSMCs, and increase uterine contraction. Moreover, blocking the BK_{Ca} channel before or after LPS addition decreases channel activity on the cell membrane and contraction of the uterus. These results indicate that BK_{Ca} channels are involved in both spontaneous uterine contraction and LPS-stimulated uterine contraction during late pregnancy in the mouse.

LPS, an endotoxin present in gram-negative bacterial cell walls, binds to TLR4 and results in a cascade of inflammatory responses in many diseases. Previous studies reported that intraperitoneal injection of LPS in pregnant Sprague Dawley rats increased the inflammatory cytokines and chemokines in serum and placenta, induced leukocytes infiltration into the chorionic plate, and led to fetal growth restriction or loss [34–36]. However, it was unclear if LPS directly promotes uterine contraction, in addition to modulating the inflammatory environment. While some early studies reported that LPS and some inflammatory cytokines could not acutely stimulate uterine contraction in vitro [37, 38], several more recent studies suggested that LPS promotes contractions [39, 40]. For example, in vitro studies using collagen gel contraction assays have shown that LPS exerts some long-term effects, which could stimulate the contraction of immortalized and primary human myometrial cells [23, 41]; in vivo studies by Sugawara and Gracious Ross suggest that injection of LPS might enhance uterine contraction in mice and rats [42, 43].

Our present study extends these observations using tension recording and reveals that LPS has an immediate and cumulative effect on uterine contraction. LPS (20 μg/ml) continually increased the amplitude of contractions in the first hour after LPS administration although there was no significant difference in frequency. This indicates that LPS has quick acting effects on uterine contraction, in addition to its known longer lasting effects via NF-κB pathway and Rho/ROCK pathway [23, 41]. To date, the direct effects of LPS on uterine contraction were only reported by Yellon's team in pregnant C3H/HeN mice [39]; however, the contribution of immune cells to uterine contraction was only suggested. Here, we show that TLR4 expression is not restricted to immune cells and is also expressed in the myometrium. Myometrial expression of TLR4 allows for a direct and rapid effect of LPS on MSMC contraction. This rapid response to LPS indicates that the effect of LPS is likely mediated through ion channels which can induce contractions much faster than transcriptional-based signaling pathways [32].

As one of the most abundant potassium channels in myometrial tissue [10–12], BK_{Ca} has been proposed, by some, to play an essential role in maintaining uterine quiescence throughout pregnancy. Inhibition of BK_{Ca} has been reported to depolarized MSMCs and increased myometrial contractility [15]. However, the effectiveness of BK_{Ca} channel blockers and openers has been questioned [17, 18, 44]. To confirm the role of BK_{Ca} in uterine contraction, we treated uterine strips from near-term mice with the BK_{Ca} inhibitor paxilline and found that uterine contraction gradually decreased. The similar inhibitory effect occurred even if we added LPS before the application of paxilline. Namely, inhibiting BK_{Ca} channels decreased uterine contraction, instead of increasing contractility as was previously reported [14]. This difference may be due to the different species (human vs mouse), different sampling sites (lower uterine segment vs cervical end of the uterus), different conditions (noninflammatory vs inflammatory), or different observation time. This also raises the possibility that BK_{Ca} channels functions differently in the mouse and human myometrium as we suggest in NALCN (sodium leak channel, nonselective), although further studies are needed to test the hypothesis [45].

To further investigate the relationship between LPS and BK_{Ca} channels and their involvement in the enhancement of contraction, single channel membrane currents were recorded in mMSMCs isolated from the myometrium of pregnant mice. Our results showed that the membrane currents of mMSMCs at a resting membrane levels (−60 mV) were very low but could be immediately and dramatically increased by LPS. More importantly, these LPS-stimulated membrane currents were decreased by BK_{Ca} inhibitors paxilline and iberiotoxin indicating that these currents are carried through BK_{Ca} channels.

LPS has been reported to activate BK_{Ca} via several mechanisms if different cell types. For example, LPS can rapidly activate BK_{Ca} channels in vascular smooth muscle cells, bladder umbrella cells, and microglia, possibly via a nitric oxide-like pathway or activation of protein kinase A [46–49]. LPS was also reported to induce the activation of murine microglia via rapidly stimulating membrane BK_{Ca} currents [49]. However, the short time effect of LPS on BK_{Ca} membrane currents in mMSMCs had not been evaluated. Our present study reveals

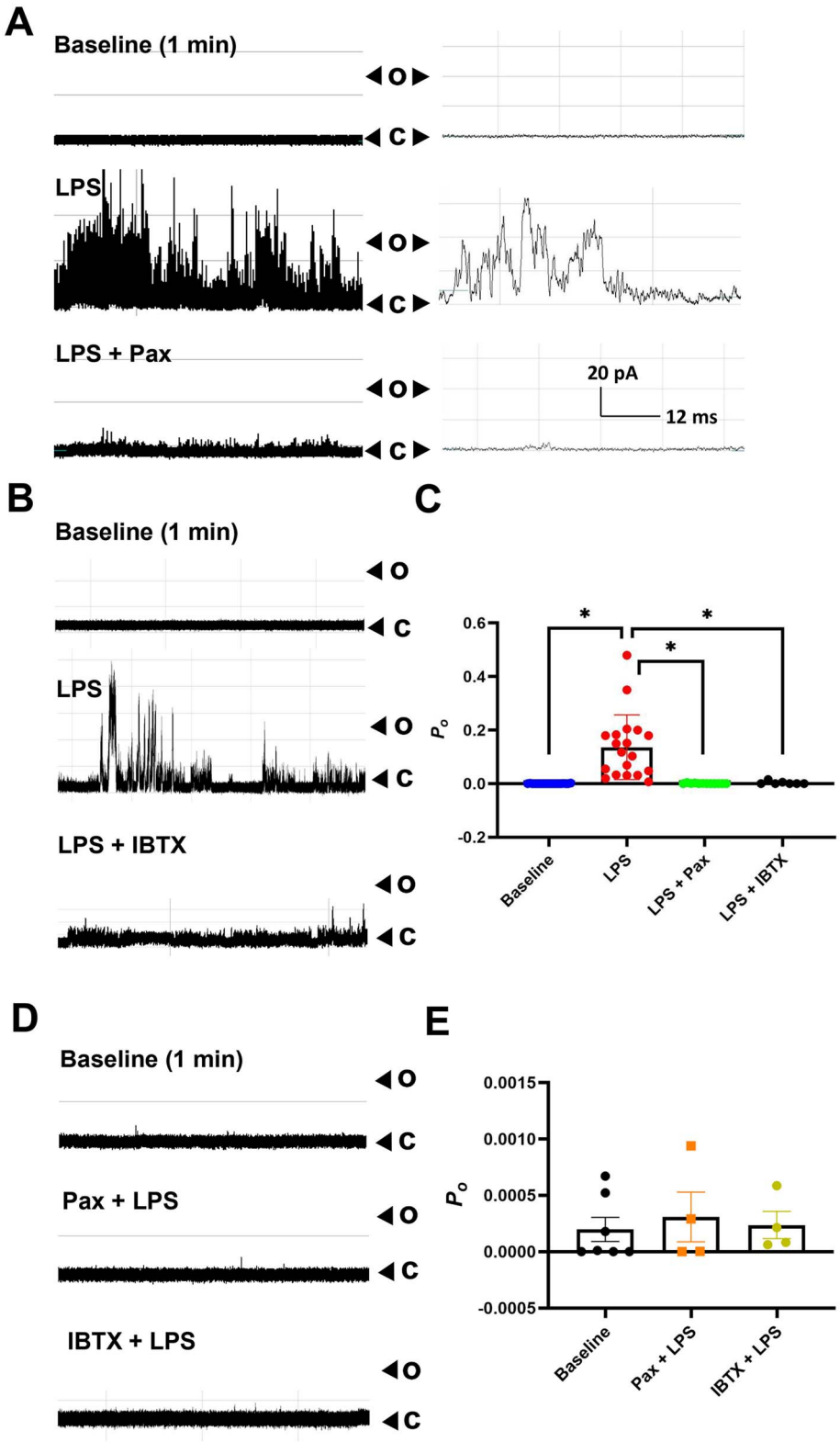

Figure 4. BK_{Ca} activity is stimulated by LPS. Representative cell-attached patch-clamp recordings at a holding potential of −60 mV in mMSMCs showing channel activity during a 1-min recording (A and B, left), and a zoomed in 60-ms recording (A, right). Recordings where taken before (baseline) and after application of LPS (10 ng/ml), and after application of paxilline (Pax, 20 μM) or iberiotoxin (IBTX, 10 nM). Arrowheads indicate closed (C) and open (O) states of the channels. (C) The single-channel open probability (P_o) of cells from A and B were calculated and compared. (D) Representative cell-attached patch-clamp recordings show channel activity during a 1-min recordings. Recordings included before (baseline) and after application of LPS post-paxilline (Pax+ LPS) or after application of LPS post-IBTX (IBTX + LPS). (E) P_o of cells from D were calculated and compared ($P > 0.050$). Data are presented as mean ± SEM from 4 to 19 independent experiments. *, $P < 0.050$.

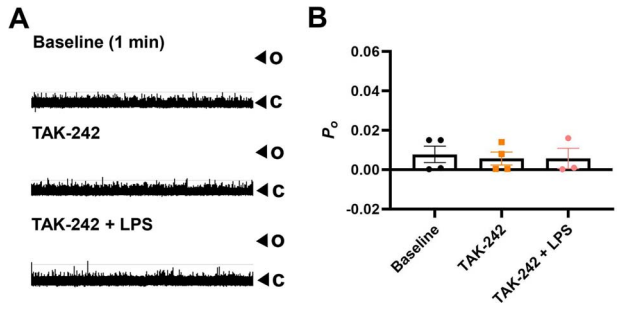

Figure 5. Blocking TLR4 with TAK-242 prevented LPS-induced BK_{Ca} membrane currents. (A) Representative cell-attached patch-clamp recordings show channel activity during a 1-min recordings. Recordings included before (baseline) and after application of TAK-242 (1 μM), and after application of LPS post-TAK-242 (TAK-242 + LPS). (B) P_o of samples from A were calculated and compared ($P > 0.050$). Data are presented as mean ± SEM from 3 to 4 independent experiments.

that LPS could immediately and dramatically stimulate BK_{Ca} membrane currents through TLR4. If we block the BK_{Ca} channels with paxilline or iberiotoxin 10 min before application of LPS or block TLR4 with TAK-242 before application of LPS, LPS was no longer able to significantly enhanced the membrane currents as determined by open-state probability.

Altogether, combining our current study with other data in the field, we speculate that there is low BK_{Ca} channel activity and Ca^{2+} influx through voltage-gated Ca^{2+} channels at basal states. However, in response to LPS, our data show that TLR4 is activated, resulting in BK_{Ca} channels opening, evoking K^+ efflux from the cell. This rapid response may be a direct reaction of BK_{Ca} channels to LPS, or it could be secondary to Ca^{2+} entering the cytosol from extracellular space or sarcoplasmic reticulum, among other sources. In this dynamic ionic equilibrium, the increase in intracellular Ca^{2+} ($[Ca^{2+}]_i$) activates Ca^{2+}-calmodulin, myosin light chain kinase, and the actomyosin machinery, triggering the escalating contraction of uterus [7, 50–52] Simultaneously, LPS may upregulate pro-inflammatory cytokines (IL-1β, IL-6, TNF-α, CCL-2), prostaglandins and uterine contraction-associated proteins (oxytocin receptor, connexin 43, prostaglandin $F_{2\alpha}$ receptor, cyclooxygenase-2) via NF-κB, p38 MAPK, Rho/ROCK, and cAMP-dependent signaling pathways [23, 53, 54], activating even stronger uterine contractions. Although not measured in this study, there is evidence that continuous contraction and relaxation of uterus may lead to brief repeated cycles of hypoxia and reoxygenation, which can accelerate some endogenous damage-associated molecular patterns releasing, promoting inflammation, and resulting in stronger uterine contractions and labor [31, 55]. It is worth mentioning that LPS can also stimulate infiltrated immune cells to produce more prostaglandins, matrix metalloproteinases, and pro-inflammatory cytokines, which can cooperate with MSMCs to promote uterine contraction [56–59].

In summary, our study establishes an LPS-stimulated uterine contraction model in vitro and demonstrates that BK_{Ca} channels are involved in both spontaneous and LPS-stimulated uterine contraction in mice during late gestation. These findings extend our understanding of the importance of the BK_{Ca} channels and the link between inflammation, BK_{Ca} channels, and uterine contractile activity in mice during late gestation. Further elucidation of the mechanisms may provide new insights into potential strategies to regulate uterine contraction.

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Supplementary material

Supplementary material is available at *BIOLRE* online.

Authors' contributions

Sarah K. England and Junjie Bao designed research; Junjie Bao, Xiaofeng Ma and Ronald McCarthy performed research; Junjie Bao and Xiaofeng Ma analyzed data; Junjie Bao wrote the first draft of the manuscript, and Sarah K. England, Xiaofeng Ma, Lindsey N. Kent, and Monali Wakle-Prabagaran revised the manuscript. All authors read and approved the final version. The authors declare that all data were generated in-house and that no paper mill was used.

Conflict of interest: The authors declare no competing interests.

Data availability

All original data are available upon request to the corresponding author. There are no large databases associated with this work.

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