CyPPA, a positive modulator of small-conductance Ca\(^{2+}\)-activated K\(^+\) channels, inhibits phasic uterine contractions and delays preterm birth in mice

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CyPPA, a positive modulator of small-conductance Ca\(^{2+}\)-activated K\(^+\) channels, inhibits phasic uterine contractions and delays preterm birth in mice. Am J Physiol Cell Physiol 301: C1027–C1035, 2011. First published July 27, 2011; doi:10.1152/ajpcell.00082.2011.—Organized uterine contractions, including those necessary for parturition, are dependent on calcium entry through voltage-gated calcium channels in myometrial smooth muscle cells. Recent evidence suggests that small-conductance Ca\(^{2+}\)-activated potassium channels (KCa2), specifically isoforms KCa2.2 and 2.3, may control these contractions through negative feedback regulation of Ca\(^{2+}\) entry. We tested whether selective pharmacologic activation of KCa2.2/2.3 channels might depress uterine contractions, providing a new strategy for preterm labor intervention. Western blot analysis and immunofluorescence microscopy revealed expression of both KCa2.2 and KCa2.3 in the myometrium of nonpregnant (NP) and pregnant (gestation day 10 and 16; D10 and D16, respectively) mice. Spontaneous phasic contractions of isolated NP, D10, and D16 uterine strips were all suppressed by the KCa2.2/2.3-selective activator CyPPA in a concentration-dependent manner. This effect was antagonized by the selective KCa2.2 inhibitor apamin. Whereas CyPPA sensitivity was reduced in D10 and D16 versus NP strips (pIC\(_{50}\) 5.33 ± 0.09, 4.64 ± 0.03, 4.72 ± 0.10, respectively), all contractions were abolished between 30 and 60 \(\mu\)M. Blunted contractions were associated with CyPPA depression of spontaneous Ca\(^{2+}\) events in myometrial smooth muscle bundles. Augmentation of uterine contractions with oxytocin or prostaglandin F2a did not reduce CyPPA sensitivity or efficacy. Finally, in an RU486-induced preterm labor model, CyPPA significantly delayed time to delivery by 3.4 h and caused a 2.5-fold increase in pup retention. These data indicate that pharmacologic stimulation of myometrial KCa2.2/2.3 channels effectively suppresses Ca\(^{2+}\)-mediated uterine contractions and delays preterm birth in mice, supporting the potential utility of this approach in tocolytic therapies.

KCa channels; tocolytic therapy; myometrium

IN THE UTERUS, L-type voltage-gated calcium channels (VGCCs) are essential for the phasic contractions of labor that result in birth. Myocyte membrane depolarization opens VGCCs, allowing Ca\(^{2+}\) influx from the extracellular space and elicit concerted periodic contractions (9, 12, 24, 27, 31, 32). Indeed, inhibition of VGCCs using dihydropyridines, such as nifedipine, is a mainstay of current clinical intervention for preterm labor in the United States. While calcium channel blockers are generally considered safer and more effective than many other tocolytics, they typically only delay birth up to 2 days, allowing time for maturation of fetal lungs through administration of a corticosteroid (3, 21, 29). In addition, because VGCCs are major conduits of Ca\(^{2+}\) entry in vascular smooth muscle, therapeutic doses of dihydropyridines against uterine contractions can result in unacceptable decreases in maternal blood pressure (3, 16, 33).

Recent efforts have explored alternate means of controlling VGCC-driven contractions. In particular, myometrial K\(^+\) channels have emerged as promising targets (7, 14, 23). In the myometrium, stimulation of these channels leads to K\(^+\) efflux and membrane potential hyperpolarization, reducing VGCC activity (37). Multiple types of K\(^+\) channels have been implicated in uterine function, including voltage-gated (K\(_{\text{v}}\)), Ca\(^{2+}\)-activated (K\(_{\text{Ca}}\)), and ATP-sensitive (K\(_{\text{ATP}}\)) forms (6, 17). Large-conductance KCa channels (BK or KCa1.1) are expressed in the myometrium although their direct functional influence on phasic uterine contractions remains dubious (1). Increasing interest has centered on a particular class of small-conductance Ca\(^{2+}\)-activated K\(^+\) channels (SK or KCa2). Unlike KCa1.1 channels that are stimulated by both depolarization and micromolar concentrations of intracellular Ca\(^{2+}\) (34), KCa2 channels are voltage independent and exhibit highly sensitive Ca\(^{2+}\)-dependent activation (half-maximal at 300 nM) (39). Thus, these channels are well suited to provide responsive feedback control of Ca\(^{2+}\) and hence contraction in the myometrium.

The KCa2 channel family is composed of KCa2.1, -2.2, and -2.3 isoforms (19). Inhibition of this family of channels with the bee venom toxin apamin increases contractile force in isolated uterine strips (7, 23), indicating their direct functional impact in the myometrium. Several studies point to KCa2.3 activity playing a particularly important role in myometrial physiology (4, 7, 23, 25). In genetically altered SK3\(^{\text{T/T}}\) mice, the overexpression of KCa2.3 channels greatly depresses uterine contractions (7) and leads to dystolic labor, despite otherwise normal gestation (4). Doxycycline-directed downregulation of KCa2.3 expression in these animals increases uterine contractions and restores normal parturition (4). More recently, a study from Pierce et al. (25) reported that SK3\(^{\text{T/T}}\) mice are protected from preterm labor, and protection is lost when KCa2.3 expression is suppressed. KCa2.1 and 2.2 expression has also been reported in the uterus, and recent data from pregnant rats suggest that expression of KCa2.2 may increase over the course of gestation (23). Specific functional contributions of KCa2.1 and -2.2, if any, have not been elucidated. Human data are limited, but evaluation by PCR has indicated that mRNA for all three KCa2 isoforms is present in human uterus (22), with KCa2.3 being substantially expressed in myometrium (10).

Overall, available data suggest that KCa2.3, and possibly KCa2.2, may be pivotal in the regulation of uterine function, and that these channels might be exploited to suppress early or pathologic contractions during pregnancy. Recently, a selective KCa2.2 channel activator, cyclohexyl-[2-(3,5-dimethyl-pyrazol-1-yl)-6-methyl-pyrimidin-4-yl]-amine (CyPPA), has become available. This compound shows high selectivity for KCa2.2

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and KCa2.3 and is reported to act as a positive modulator of these channels by increasing their relative calcium sensitivity (15). Moreover, a recent study by Vick et al. (35) shows that CyPPA can be safely administered by intraperitoneal (15 mg/kg) injection and is well tolerated by mice. We surmised that CyPPA sensitization of KCa2.2/2.3 opening could substantially reduce Ca\(^{2+}\) influx and force generation in the myometrium. Here we assessed the capacity of pharmacologic KCa2.2/2.3 activation to abate uterine phasic contractions over the course of pregnancy and delay preterm birth in mice.

**MATERIALS AND METHODS**

**Animals.** Nonpregnant and timed-pregnant C57BL/6 mice were used in this study. Mice in the midpregnant state were 9 to 11 days post coitus (DPC) and labeled gestation day 10 (D10), and late-pregnant mice were DPC 15 to 17 and labeled gestation day 16 (D16); term gestation is DPC 19. Mice were euthanized with intraperitoneal injection of pentobarbital sodium (100 mg/kg) followed by thoracotomy. The abdominal cavity was opened. The uterus was excised and placed in cold (4°C) bicarbonate-buffered physiological saline solution (PBS), pH 7.4, containing (in mM) 119 NaCl, 4.7 KCl, 23 NaHCO3, 1.2 KH2PO4, 0.026 EDTA, 1.2 MgSO4, 2 CaCl2, and 10.5 glucose. All animal procedures were approved by the University of South Alabama Institutional Animal Care and Use Committee.

**Western blot analysis.** Sections of endometrium-free uterine tissue (~3 mm\(^2\) of antimesometrial tissue most proximal to the oviduct) were frozen in liquid nitrogen and pulverized. Lysate buffer containing (in mM) 20 Tris·HCl (pH 7.5), 150 NaCl, 1 Na2EDTA, 1 EGTA, 1% Triton X-100, 2.5 sodium pyrophosphate, 1 β-glycerophosphate, 1 Na3VO4, 1 μg/ml leupeptin, and 1 PMSF was added to pulverized tissues. Tissues were homogenized in a Kontes glass-glass tissue grinder on ice. Tissue lysates were clarified by centrifugation at 10,000 g for 15 min at 4°C. Total protein was determined by the detergent-compatible protein assay method (Bio-Rad). Clarified uterine homogenates were subjected to SDS-polyacrylamide gel (10%) electrophoresis (25 g/ml total protein/lane) and transferred to a nitrocellulose membrane. Membranes were blocked in 5% nonfat milk in PBS with 0.1% Tween 20. Membranes were then exposed to primary antibodies overnight. Primary antibodies were KCa2.2 (Millipore; 1:600), KCa2.3 (Millipore; 1:250), and GAPDH (Cell Signaling; 1:3,000). Primary antibody selectivity was evaluated with positive controls (Fig. 1) and blocking peptides (data not shown). After exposure to anti-rabbit-horseradish peroxidase secondary antibody (Cell Signaling 1:3,000), the membranes were visualized by chemiluminescence (SuperSignal West Dura, Thermo Scientific). Densitometry was performed using Image Gauge software (version 4.0). Expression was quantified relative to GAPDH.

**Immunofluorescence.** Sections of uterine tissue were cleaned in PBS, and the endometrium was removed. Thin sections of myometrium were pinned to a nitrocellulose membrane. Membranes were blocked in 5% nonfat milk in PBS with 0.1% Tween 20. Membranes were then exposed to primary antibodies overnight. Primary antibodies were KCa2.2 (Millipore; 1:600), KCa2.3 (Millipore; 1:250), and GAPDH (Cell Signaling; 1:3,000). Primary antibody selectivity was evaluated with positive control tissue (Fig. 1) and blocking peptides (data not shown). After exposure to anti-rabbit-horseradish peroxidase secondary antibody (Cell Signaling 1:3,000), the membranes were visualized by chemiluminescence (SuperSignal West Dura, Thermo Scientific). Densitometry was performed using Image Gauge software (version 4.0). Expression was quantified relative to GAPDH.

**Isometric force recording.** Sections of uterine horn were isolated and the uterine lumen was exposed. Tissue strips were cut longitudinally to approximately 5 mm by 1 mm and mounted on a myograph apparatus (DMT 610M); one end of each strip was mounted to a force transducer and the other end was held fixed. All experiments were performed at 37°C in gassed (95% O\(_2\), 5% CO\(_2\)) PSS. Strips were stretched to their optimal lengths (L\textsubscript{o}R, ~200% and 250% of resting length for nonpregnant and pregnant strips, respectively, determined by maximal force amplitudes achieved following an incremental stretch protocol), and after a 30-min equilibration period, changes in force were recorded with Chart software. A rise >10% of the maximal 60-mM KCI-induced contraction for each individual strip was considered a contraction. Only nonzero values were included in mean frequency calculations.

**Ca\(^{2+}\) imaging.** For calcium imaging, sections of uterus were cleaned in 10 mM HEPES-buffered PSS (pH 7.4), and the endometrium was removed. Thin sections of myometrium were pinned to small silicone blocks (stretched by ~100 or 150% of resting length for nonpregnant and pregnant) and loaded with 10 μM fluo-4 AM (Molecular Probes) in HEPES PSS for 40 min at room temperature. Strips were rinsed with HEPES PSS, mounted in a chamber, and imaged with a PerkinElmer RS3 spinning disk confocal microscope at 27°C. Data were acquired with Ultraview software (480 nm excitation/510 nm emission; 5 frames/s) in 10- to 12-μm-deep z-stacked frames, allowing for full-depth assessment of myometrial bundles and avoiding artifact from focal shifts during contractions. Distinct myometrial regions of interest were normalized to mean field background fluorescence (F0).

**Induction and assessment of preterm birth.** Preterm birth studies were conducted in D16 timed-pregnant mice (Charles River). RU486 was dissolved in ethanol and diluted in PBS (40%/60% vol/vol). CyPPA was dosed at 40 mg/kg in 10 ml/kg and was dissolved in Cremophor-EL (Sigma), and then diluted in PBS (5%/95%/vol/vol). The optimal dose of RU486 and the tolerance of the CyPPA dose were determined in an initial pilot study. Vehicle was Cremophor-EL/PBS. All drugs were prepared fresh and sterile-syringe filtered (0.22 μm) before administration. Mice were given 100 μg RU486 (in 100 μl) subcutaneously in the nape to induce preterm birth as previously described (9) at time 0 (T0). Beginning at 8 h (T8), dams were monitored every 1–2 h for signs of labor (vaginal bleeding, straining).

![Figure 1](http://ajpcell.physiology.org/)

**Fig. 1.** Expression of Ca\(^{2+}\)-activated potassium channels KCa2.2 and KCa2.3 in mouse uterus. **Top:** Western blots of endometrium-free uterine horn segments showing the presence of KCa2.2 and KCa2.3 in nonpregnant (NP), gestation day 10 (D10), and gestation day 16 (D16) tissues (each lane is homogenate pooled from 4–6 animals). Apparent molecular mass of each protein is listed on the left. **Bottom:** densitometry analysis of Western blot data normalized to GAPDH.
At T11 (when consistent signs of labor were visible), dams were given either vehicle or CyPPA by intraperitoneal injection and monitored every hour for delivery. Dosing was repeated at T15 and T19. Preterm birth was defined as delivery of at least one pup by T24. Dams that had not delivered by T24 were considered delivered at T24 for mean time comparison. At T24, mice were euthanized and necropsied for assessment of retained pups and placental attachment sites.

Materials. CyPPA (dissolved in DMSO for in vitro studies), prostaglandin F2α (PGF2α), oxytocin, and RU486 were purchased from Sigma (St. Louis, MO). Apamin was purchased from Genscript (Piscataway, NJ).

Data analysis. Data are reported as means ± SE, and n is number of animals for all in vitro and in vivo experiments. Data were tested for statistical differences with t-test or one-way ANOVA and post hoc Bonferroni for comparison of means where appropriate. Fetal retention was analyzed by Fisher’s exact test. Statistical significance was designated as P < 0.05.

RESULTS

KCa2.2 and KCa2.3 channel expression in mouse myometrium. To determine KCa2.2 and KCa2.3 channel levels in the uterus over gestation, we evaluated protein expression in isolated endometrium-free uterine sections from nonpregnant (NP), day 10 pregnant (D10), and day 16 pregnant (D16) mice by Western blotting (Fig. 1). Both isoforms were detected at every time point assessed. Antibody specificity was supported by positive control tissue (brain; Fig. 1A) and by separate blots employing blocking peptides for both isoforms (data not shown). Overall, KCa2.2 and KCa2.3 exhibited similar expression levels in the NP state, with KCa2.3 decreasing by ∼50% and KCa2.2 increasing by ∼100% by late gestation. Immunofluorescence labeling of intact uterine strips confirmed expression of both isoforms specifically within longitudinal smooth muscle cells of the myometrium (Fig. 2A). Secondary antibody-only controls showed no signal (Fig. 2B). Notably, we found that KCa2.2 is diffusely distributed within the cytosol of myocytes whereas KCa2.3 is highly concentrated along the cell periphery, consistent with predominant membrane localization (Fig. 2C). No obvious change in cellular distribution of the channels was noted over the course of gestation.

CyPPA suppression of phasic uterine contractions. Having identified potential targets, we next investigated the effect of CyPPA on uterine contractions by performing myography on isolated uterine strips from NP, D10, and D16 mice. All strips exhibited spontaneous phasic contractions of similar frequency, and these contractions were essentially abolished by the VGCC blocker nifedipine (98.9 ± 0.6, 99.5 ± 0.2, and 96.7 ± 2.5%, respectively, n = 3–4 per group; Fig. 3). In spontaneously contracting uterine strips from nonpregnant and pregnant mice, increasing concentrations of CyPPA had an ambiguous impact on contraction frequencies but notably decreased contraction amplitudes (Fig. 4, A and B). Contractions of all CyPPA-suppressed strips could be appreciably restored by addition of 1 µM apamin, a KCa2 channel inhibitor (Fig. 5). Overall, CyPPA responses were right-shifted in D10 and D16 strips compared with NP strips and all contractions were abolished between 30 and 60 µM (Fig. 4 and Table 1), indicating reduced CyPPA sensitivity with pregnancy but no loss of efficacy. Figure 6 shows Ca2+-dependent fluorescence measured in longitudinal myometrial bundles of pregnant (D10) mouse uterus. CyPPA treatment substantially blunted the magnitude of spontaneous periodic calcium events consis-

Fig. 2. KCa2.2 and KCa2.3 expression in smooth muscle cells of the myometrium. A: immunofluorescent staining showing the presence of KCa2.2 (red) and KCa2.3 (red) in myocyte bundles of longitudinal myometrial strips from NP, D10, and D16 mice. Nuclei are stained green (bar, 20 µm). B: secondary antibody-only control. C: single 0.25 µm-thick confocal slices through the center of D10 uterine myocytes showing diffuse distribution of KCa2.2 and predominant localization of KCa2.3 to the cell periphery (bar, 5 µm).
tent with the marked suppression of individual phasic contraction amplitudes.

Because laboring and preterm laboring uterine tissues are exposed to procontractile stimuli including the hormone oxytocin (2, 3) in vivo, we assessed the ability of CyPPA to suppress phasic contractions under these conditions. Stimulation of uterine strips with either 1 nM oxytocin or 1 μM PGF2α enhanced both phasic contraction amplitude and frequency (Fig. 7A). Application of CyPPA evoked concentration-dependent decreases in contraction amplitude, completely abolishing contractions at concentrations of 45–60 μM. CyPPA depression of agonist-enhanced contractions was effectively antagonized by apamin (Fig. 7B). Overall, prestimulation with oxytocin or PGF2α did not significantly reduce the maximal CyPPA effect in pregnant or nonpregnant strips (Table 1). In the presence of oxytocin, CyPPA responses remained relatively right-shifted in D10 strips compared with NP strips; however, responses in D16 strips were not significantly different from NP.

CyPPA-induced delay of preterm birth. To evaluate the efficacy of CyPPA as a tocolytic, we employed a preterm birth mouse model using RU486 in DPC 16 mice (9). Experimental animals were administered CyPPA at 40 mg/kg. In vehicle control animals, 100% of induced dams delivered by T20 (Fig. 8). Dams treated with CyPPA had a 3.4-h delay of preterm birth \( (P < 0.05) \) and one CyPPA-treated dam did not deliver by T24. Furthermore, 27% of fetuses were retained in control dams \( (P < 0.05) \) by two-sided Fisher’s test.

**DISCUSSION**

In the current study, we demonstrate that small-conductance \( \text{Ca}^{2+} \)-activated \( \text{K}^{+} \) channels can be pharmacologically targeted to depress uterine activity and delay preterm birth. Specifically, we provide evidence that stimulation of myometrial \( \text{K}_{\text{Ca}2.3/2.2} \) channels with the positive modulator CyPPA blocks \( \text{Ca}^{2+} \)-mediated phasic contractions in both nonpregnant and pregnant mouse uteri, and that administration of CyPPA to mice during RU486-induced preterm labor significantly delays parturition and increases fetus retention. Our findings suggest that pharmacologic \( \text{K}_{\text{Ca}2.3/2.2} \) channel activation may provide an effective tocolytic strategy for future clinical intervention.

Several recent studies have highlighted a pivotal negative feedback role for \( \text{K}_{\text{Ca}2.2} \) channels in controlling uterine excitability and have suggested their crucial impact on the progression and outcome of gestation (4, 7, 23, 25). Appreciation for the functional influence of these channels is derived largely from studies of the conditional \( \text{K}_{\text{Ca}2.3} \)-overexpressing (SK3T/T) mouse. In this model, mice exhibit drastically impaired uterine contraction magnitude, compromised parturition, and protection from induced preterm labor, all of which are corrected or prevented by antibiotic-driven \( \text{K}_{\text{Ca}2.3} \) downregulation (4, 7, 25, 26). Since targeted genetic manipulation of channel expression is not presently a feasible approach for acute clinical intervention, the current study addressed whether native myometrial \( \text{K}_{\text{Ca}2.2} \) channels might be exploited pharmacologically to amplify their functional impact and promote uterine quiescence, particularly during pregnancy. Here, we employed CyPPA because it provides the best profile of potency and selectivity for uterine \( \text{K}_{\text{Ca}2.2} \) isoforms (\( \text{K}_{\text{Ca}2.3} \) and \( \text{K}_{\text{Ca}2.2} \)) compared with other activators of this family such as EBIO and NS309 (38), both of which also target \( \text{K}_{\text{Ca}2.1} \) channels as well as related intermediate-conductance \( \text{K}_{\text{Ca}3.1} \) channels. In particular, \( \text{K}_{\text{Ca}3.1} \) channels are highly expressed in the arterial endothelium where they promote vasodilation (28), and their stimulation is preferably avoided to diminish the risk of unwanted hypotension.

We found that CyPPA suppression of phasic uterine contraction is concentration dependent, with nonpregnant tissue exhibiting greater sensitivity than pregnant tissue. Above 30 μM, CyPPA completely abolishes contractions in both pregnant and nonpregnant uterus, indicating that the functional influence of \( \text{K}_{\text{Ca}2.2} \) channels is substantial and remains recruitable over gestation. Selective block of \( \text{K}_{\text{Ca}2.2} \) channels with apamin improved or restored CyPPA-attenuated contractions in pregnant as well as nonpregnant tissues. While this antagonism does not establish a definitive mechanism of CyPPA action, it does support a functional impact of \( \text{K}_{\text{Ca}2.2} \) channel activation. Although CyPPA has been reported to inhibit tetrodotoxin-sensitive \( \text{Na}^{+} \) channels (15), we have found no effect of tetrodotoxin on phasic uterine contractions (data not shown). The cause of diminished CyPPA sensitivity during pregnancy is unclear, but may reflect altered expression/distribution of \( \text{K}_{\text{Ca}2.2} \) channels (i.e., related to a general downregulation of \( \text{K}_{\text{Ca}2.3} \)) and/or their effective coupling with voltage-gated \( \text{Ca}^{2+} \) channels during gestation. CyPPA has also been reported...
to inhibit large-conductance Ca\textsuperscript{2+} activated K\textsuperscript{+} channels (13), which might variably oppose direct K\textsubscript{Ca2} activating effects over gestation.

We show that both CyPPA-targeted isoforms, K\textsubscript{Ca2.2} and 2.3, are expressed in murine myometrium over the course of gestation. The current study did not clearly delineate relative contributions of K\textsubscript{Ca2.2} and 2.3 although K\textsubscript{Ca2.3} channels were found to be highly localized in the cell periphery, indicative of their function as plasma membrane ion channels. Although overexpression and suppression of K\textsubscript{Ca2.2} levels in genetically altered mice have been linked to pup viability (5, 13, 20), specific effects on gestation and parturition have not been reported. In fact, following suppression of K\textsubscript{Ca2.3} expression, the global K\textsubscript{Ca2} blocker apamin does not significantly alter uterine contractions (7), questioning any functional impact of other isoforms. Protein expression data in gestational human myometrium are lacking. Nevertheless, mRNA data suggest little or no K\textsubscript{Ca2.1} and K\textsubscript{Ca2.2} expression in the nonpregnant state (10) while K\textsubscript{Ca2.3} is present and persists, albeit at a reduced level, at term (22). Overall, our data support the concept that K\textsubscript{Ca2.3} expression may be particularly pivotal in determining the uterine effects of K\textsubscript{Ca2}-targeting drugs and related therapeutic windows.

The major effect of CyPPA is a graded reduction of uterine contraction amplitude with a minimal net impact on contraction frequency. Our data are consistent with a mechanism in

![Figure 4](http://ajpcell.physiology.org/)
which increasing KCa2 channel sensitization allows for graded potentiation of negative Ca2+ feedback in the smooth muscle of the myometrium. This implies that the very intracellular Ca2+ events that trigger contractions become increasingly self-limiting in the presence of CyPPA, restricting the magnitude of individual phasic events. Indeed, we observed marked truncation of the concerted Ca2+ bursts within distinct smooth muscle bundles in the myometrium. The similar blunting of Ca2+ and force amplitudes by CyPPA with essential preservation of frequency suggests that KCa2 channels may play little role in regulating inherent pacemaker cells that drive the procontractile action potentials but rather effectively mute bulk Ca2+ entry in the myometrium once initiated. These findings suggest that the periodicity and force of uterine contractions may be modulated independently, possibly providing new angles for intervention. Also, although the current study focused on longitudinal muscle, we suggest that influences of KCa2 activation on the circular muscle of the myometrium could additionally impact the directional force and coordination of uterine contractions.

We considered that, during gestation, agonists such as oxytocin and prostaglandins promote intense contractions that might be more refractory to KCa2 activation. However, we

### Table 1. Summary of CyPPA-induced inhibition of uterine contraction amplitudes

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<th>NP</th>
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<td></td>
<td>pIC50</td>
<td>Max, %</td>
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<tr>
<td>Basal</td>
<td>5.33 ± 0.09</td>
<td>100</td>
<td>4.64 ± 0.03*</td>
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<tr>
<td>Oxytocin pretreated</td>
<td>5.09 ± 0.07</td>
<td>100</td>
<td>4.71 ± 0.08*</td>
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<tr>
<td>PGF2α pretreated</td>
<td>5.25 ± 0.04</td>
<td>100</td>
<td>4.56 ± 0.04*</td>
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Values for pIC50 are mean −log [CyPPA] ± SE (n = 4–6 animals per time point). Basal refers to spontaneous contractions in the absence of agonist. Maximal effect (Max) is the percent decrease in contraction amplitude after CyPPA treatment. NP, nonpregnant; D10, day 10 gestation; D16, day 16 gestation. *P < 0.05 compared with corresponding NP.
found that although both oxytocin and PGF2α robustly increase uterine activity in pregnant and nonpregnant tissues, these amplified contractions remain fully susceptible to CyPPA with no relative loss of CyPPA sensitivity or effect. Interestingly, in the presence of oxytocin, CyPPA responses at D16 are not significantly right-shifted from NP (see Table 1), suggesting a possible increasing KCa2 influence in the presence of this agonist. Whether this trend reflects enhanced oxytocin-specific Ca2+ targeting of KCa2 channels remains an interesting question for future study. At any rate, our findings show continued effectiveness of CyPPA in the face of advancing pregnancy and in the presence of procontractile stimuli.

Finally, we evaluated the potential tocolytic action of CyPPA. Potential side effects and off-target effects of KCa2-modifying drugs should be considered in the context of in vivo use. KCa2 channels are expressed in tissues outside the uterus including gastrointestinal and urinary bladder smooth muscle (10, 36) as well as neuronal and endothelial cells. Potential effects via the placenta or fetus are unknown. Notably, although KCa2.3 channels in the vascular endothelium exert a modulatory influence on vascular tone, we previously showed that persistent KCa2.3 overexpression had no impact on blood pressure in mice (30). Consistent with recent findings (35), we found transient quiescence to be the only notable side effect in mice following intraperitoneal CyPPA injection. Overall, repeated bolus administration of CyPPA incurred no obvious deleterious effects in mice, supporting the expediency of its use in vivo. Here, we specifically assessed the ability of CyPPA to delay preterm parturition in mice induced with the progesterone receptor antagonist RU486. This method reliably induced preterm birth because 100% of control (RU486 vehicle) animals delivered within 20 h of injection, concordant with previous studies utilizing this model (8, 11, 18, 25, 26). We employed DPC 16 mice since induction at earlier gestation times was associated with pup resorption in pilot studies. To directly evaluate its potential in preterm labor intervention, we administered CyPPA only after consistent signs of labor had begun, 11 h after RU486 injection (T11). This time point correlates with a distinct increase in intrauterine pressure as reported by Pierce and colleagues (26). In the 13-h experimen-

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**Fig. 7. Effect of CyPPA on agonist-enhanced contractions of uterine strips from pregnant mice. A:** representative force recordings from D10 uterine strips showing sustained enhancement of phasic contractions (top) in response to 1 nM oxytocin (left) or 1 μM PGF2α (right) and subsequent concentration-dependent depression of contractions (bottom) by CyPPA (arrows indicate CyPPA concentrations in μM). **B:** recordings showing the effect of apamin (1 μM) on oxytocin or PGF2α-stimulated, CyPPA-suppressed contractions.

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**Fig. 8. Effect of CyPPA on first-pup delivery in preterm laboring mice.** D16 dams received RU486 (at time 0, T0) to induce preterm labor and were subsequently treated with vehicle (●, n = 8) or CyPPA (▲, n = 7) at 11 h (T11), 15 h (T15), and 19 h (T19). Data represent percentage of dams delivering at least one pup by the indicated time. Mean delivery time for CyPPA treated dams was 3.4 h later than vehicle treated (P < 0.05).
nal treatment window assessed (T11–T24), CyPPA significantly delayed parturition by an average of 3.4 h and increased the number of pups retained in the uterus over 2.5-fold. One limitation of the current study was the bolus administration of drug over the limited time course evaluated. Future study of earlier prophylactic administration and continual infusion is warranted. Also, levels of KCa2.2 expression during RU486 treatments are unknown and the impact of CyPPA on uterine contractions in vivo was not directly measured. Nevertheless, our findings demonstrate a clear effect of CyPPA as an acute intervention during active preterm labor.

In summary, this study provides evidence that pharmacological stimulation of myometrial KCa2.2 channels with the agent CyPPA effectively depresses uterine contractions in mice. Administered in vivo, CyPPA delays preterm parturition and increases fetuses retention. Our data support further development of KCa2 targeting drugs and suggest that this approach, alone or in combination with current tocolytics, may provide a novel therapeutic strategy, allowing for targeted and titratable intervention to control preterm labor and birth.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

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