Prolonged stretching in the late-late pregnant rat uterus inhibits contractions through potassium channel TREK-1

Zongzhi Yin
The first affiliated hospital of anhui medical university

Yun Li
the first affiliated hospital of anhui medical university

Dan Li
the second affiliated hospital of anhui medical university

Jingjing Su
the first affiliated hospital of anhui medical university

Bing Shen
department of physiology, anhui medical university

Yuanyuan Yang
the first affiliated hospital of anhui medical university

Yunxia Cao (✉ caoyunxia6@126.com)

Research article

Keywords: stretching, potassium channel TREK-1, uterine contraction, pregnant, rat

DOI: https://doi.org/10.21203/rs.3.rs-30853/v1

License: © This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

Background

Prolonged stretching can inhibit myometrial contractions in the nonpregnant and the mid- and late-term pregnant uteri of rats and humans through potassium channel TREK-1. What happens in the pregnant uterus close to parturition remains unknown.

Methods

Uterine tissues from late-late pregnant rats (day 20–21 of pregnancy) were isolated, and myometrial strips were prepared for isometric contraction measurements. We compared the oxytocin-induced contractions of myometrial strips treated with different stretch times and doses. Then, we used the potassium channel TREK-1 inhibitor L-methionine and TREK-1 agonist arachidonic acid to determine whether the changes caused by prolonged stretching involved changes in TREK-1 activity.

Results

Prolonged stretching caused relaxation of the myometrial strips that were close to parturition. Additionally, TREK-1 inhibition partly reversed the myometrial relaxation caused by prolonged stretching of the late-late pregnant rat uterus, while TREK-1 activation resulted in a dramatic myometrial contraction change.

Conclusions

Prolonged stretching can inhibit myometrial contractility and induce myometrial relaxation in late-late pregnancy. This contractile inhibition is partly due to functional upregulation of potassium channel TREK-1.

Background

During pregnancy, the uterus dramatically expands in volume to provide enough space for the growing fetus [1]. To guarantee sufficient time for the development of the fetus before labor, mechanical quiescence of the myometrium is needed to decrease the muscle excitability caused by hypertrophy and distension of the myometrium during pregnancy [2]. Once the myometrium is stimulated at term, it transitions into an excitable state and becomes one of the strongest muscles to facilitate birth [3]. The mechanisms that facilitate uterine quiescence during pregnancy and enhance contractility at term are not fully understood.
Previous studies have shown that during pregnancy, uterine contractility dramatically decreases [4]. Prolonged stretching of the nonpregnant and mid-term pregnant rat uterus inhibits myometrial contractions. Similarly, during the late stage of a term pregnancy, human uterine contractility decreases compared to that of a preterm pregnancy. Additionally, prolonged stretching of the preterm pregnant uterus can inhibit contractions to a level that is similar to that of the term pregnant uterus [5]. All of these data show that stretching can inhibit myometrial contractions in the nonpregnant and mid- and late-term pregnant uteri of rats and humans. Whether the myometrium remains sensitive to stretching close to parturition is unclear.

Increases in intracellular calcium (Ca$^{2+}$) can trigger uterine contractions, especially through voltage-gated channels. The decline in intracellular Ca$^{2+}$ caused by membrane hyperpolarization and activation of different types of potassium (K$^+$) channels maintains smooth muscle relaxation [6, 7]. A previous study has shown that potassium channels play a vital role in uterine functions, and several of these channels have been proposed to maintain uterine quiescence during pregnancy up to full term [8]. The TREK-1 channel, a stretch-activated tetraethyl ammonium-insensitive potassium current in smooth muscles that has been isolated from human and rat myometrial tissues, decreases myometrial smooth muscle excitability through its contributions toward returning the depolarized cell to a more negative resting potential and maintaining the resting cell near the K$^+$ equilibrium potential [9, 10].

In the human pregnant uterus, higher TREK-1 expression is observed in term and twin pregnancies. Prolonged stretching of the preterm human uterus increases TREK-1 expression [5]. The sex hormone progesterone decreases uterus contractions in late pregnant rats partly through TREK-1 channels [11]. Whether prolonged stretching of the rat uterus close to parturition can still stimulate TREK-1 function remains unknown.

In the present research, we used the rat uterus at 20–21 days of pregnancy to investigate (1) the contractility differences of the pregnant uterus close to parturition (late-late pregnancy) under prolonged stretching and (2) the TREK-1 function in processing prolonged stretching with late-late pregnant uterine contractions.

Methods

Tissue preparation

The study was conducted on late-late pregnant Sprague-Dawley rats purchased from Animal Laboratory Center of Anhui Medical University (aged 12 weeks, weighing 300 to 350 gram (g) when pregnant). Because the last day of pregnancy in rats is day 21 or day 22, we defined late-late pregnancy as 20–21 days of gestation in rats, which is close to parturition but without the start of uterine contractions. The rats were housed in the animal facility and maintained on standard rat chow and tap water ad libitum in a 12:12-h light-dark cycle. 10 rats were used in stretch group, 10 rats were used in arachidonic acid group and 10 rats were used in L- methionine. The animals were euthanized by inhalation of 100% carbon
dioxide (CO\textsubscript{2}) in a closed chamber, and death was indicated by cessation of breathing and heartbeats. The pregnant rat uterus was rapidly excised, and connected tissues were cleaned under microscopic visualization followed by the removal of the fetus and placenta. The uterus was immediately immersed in Krebs solution for further dissection. Because oxytocin is also synthesized within the decidual tissues immediately adjacent to the myometrium, the decidua was removed from the myometrial strips prior to myographic assessment. From each rat, longitudinal uterine strips (3 mm × 7 mm, width × length) were isolated and suspended vertically in four individual, temperature-controlled organ bath chambers containing 5 milliliters (ml) of Krebs solution bubbled with 95% O\textsubscript{2}/5% CO\textsubscript{2} at 37°C. The end of each uterine strip was attached to a fixed glass hook at the bottom of the organ bath chamber, and the other end was hooked to an isometric force transducer (6240 biological treatment systems). Changes in isometric contractions were recorded.

All procedures were performed in accordance with the guidelines of the Institutional Animal Care and Use Ethics Committee at the First Affiliated Hospital of Anhui Medical University, Hefei, China (20170210).

**Isometric contraction measurement**

The uterine strips of pregnant rats were stretched under the control of 2 g basal tension to equilibrate for 1 h with constant Krebs solution and stimulated twice with 96 mM potassium chloride (KCl) to obtain a maximal response. Each control KCl contraction was followed by three washes in Krebs solution, 10 min each. To demonstrate the effect of prolonged stretching on myometrial contractions, the uterine strips were subjected to either control 2 g basal tension or high 8 g tension for 18 h in tissue culture medium. On the next day, the bathing solution was changed to Krebs solution, and the oxytocin contraction was measured.

To determine whether TREK-1 was involved in the uterine contraction changes associated with prolonged stretching, contraction experiments were repeated in the presence of TRKE-1 inhibitor L-methionine (Sigma-Aldrich) and activator arachidonic acid (Sigma-Aldrich). The area under the curve (AUC) was measured at time 0 and was subtracted from the AUC measured after 5 min of application of each oxytocin concentration by the R1640 data analysis system (Chengdu, China). The AUC representing total contractions was also normalized to the weight of the myometrial strip and is presented as the AUC per gram of tissue. The oxytocin-induced contraction response was calculated for 20, 40, and 60 min.

**Solutions and drugs**

Normal Krebs solution contained (in mM): 120 NaCl, 5.9 KCl, 25 NaHCO\textsubscript{3}, 1.2 NaH2PO\textsubscript{4}, 11.5 dextrose, 2.5 CaCl\textsubscript{2}, and 1.2 MgCl\textsubscript{2} (Sigma). The pH of the Krebs solution was 7.3–7.4 when bubbled with 95% O\textsubscript{2} 5% CO\textsubscript{2} at 37.0 ± 0.5 °C. A high KCl solution (96 mM) was prepared similarly to normal Krebs but with equimolar substitution of NaCl with KCl. Oxytocin (Shanghai Hefeng Pharmaceutical Company, China) was dissolved in deionized water. A stock solution of L-methionine (Sigma, 10^{-1} M) was prepared in deionized water. A stock solution of arachidonic acid (Sigma, 10^{-3} M) was prepared in ethanol. The final concentration of ethanol was less than 0.1% and had no effect on uterine contractions. The tissue culture
medium used for prolonged incubation of the uterine strips for 16 h comprised Minimum Essential Medium supplemented with penicillin, streptomycin, and amphotericin B (Gibco/Invitrogen, Grand Island, NY). All other chemicals were of reagent grade or better.

**Statistical analysis**

Data were analyzed and presented as the mean ± SEM, with “n” representing the number of subjects per group. For uterine contraction experiments, individual concentration-contraction curves were constructed. Data were first analyzed using ANOVA of tissue stretching (control 2 g basal tension vs 8 g stretching) and tissue treatment (treated with arachidonic acid or L-methionine vs nontreated control tissues). When a significant difference was observed, the data were further analyzed using Bonferroni's post hoc test for multiple comparisons. Student's unpaired t-test was used for comparisons of two means. Differences were considered statistically significant if P < 0.05.

**Results**

**Oxytocin-induced contractions in the late-late pregnant rat uterus under prolonged stretching**

In the isolated late-late pregnant rat uterine strips, oxytocin caused an increase in the frequency and amplitude of uterine contractions that did not return to baseline, leaving a measurable maintained response above baseline. Oxytocin caused a concentration-dependent increase in the maintained contractions, and the contractions of the rat uterine strips peaked at $10^{-7}$ M oxytocin, as previously described. The oxytocin-induced contractions decreased in the uterine strips under prolonged 8 g stretching compared to those in the control tissues under control 2 g basal tension (Fig. 1a). The cumulative data suggested that the oxytocin-induced maintained contractions in the AUC/g tissue were significantly reduced in tissues under 8 g stretching compared to control tissues under 2 g basal tension for 20 min, 40 min and 60 min, respectively (Fig. 1b).

**Effect of TREK-1 activator on oxytocin-induced uterine contractions**

For the uterine strips from the late-late pregnant rats and those that were precontracted with oxytocin ($10^{-7}$ M), treatment with the vehicle was associated with a slight time-dependent decline in the AUC/g tissue, representing total uterine contractions, and treatment with activator of TREK-1 appeared to further modulate oxytocin contractions (Fig. 2a). Cumulative data revealed that the TREK-1 activator arachidonic acid ($10^{-5}$ M) caused a further decline in the AUC/g tissue, representing total contractions of the uterine strips without prolonged stretching for 20 min, 40 min and 60 min, respectively. However, arachidonic acid caused no further decline in contractions compared to the uterine strips stretched under 8 g stretching for 18 h (Fig. 2b, c, d, e).
Effect of TREK-1 inhibitor on oxytocin-induced uterine contractions

For the uterine strips from the late-late pregnant rats and those that were precontracted with oxytocin (10^{-7} M), treatment with the TREK-1 blocker L-methionine (1 mM) minimized the decline in uterine contractions (Fig. 3a). Treatment with TREK-1 blocker L-methionine significantly enhanced the AUC/g tissue, representing total contractions of the uterine strips with prolonged 18 h stretching for 20 min, 40 min and 60 min, respectively (Fig. 3b, c, d, e).

Discussion

The present study of the late-late pregnant rat uteri showed that (1) uterine contractions are further reduced under 8 g stretching of the uterine wall for 18 h; (2) TREK-1 activation reduces uterine contractions in late-late pregnant rats; and (3) TREK-1 blockade reverses the decrease in uterine contractions, particularly during exposure to prolonged stretching.

The regulation of uterine contractions is critical for normal healthy pregnancy and preterm labor [12]. During pregnancy, the uterus expands progressively and proportionately to increases in fetal size. In full-term pregnancy, the uterine stretching imposed by the fully developed fetus reaches a threshold level to initiate labor. We evaluated late-late pregnant uterus contractions and found that prolonged stretching of the uterus could significantly inhibit uterine contractions. The results showed that the myometrial relaxation system continued to function late in gestation, when parturition was very close, at which point spontaneous contractions would start. The quiescent fluctuation of the myometrium could be induced in the parturient woman. These observations are consistent with the findings that parturition is triggered at a specific point.

Previous studies have shown that threshold increases in uterine wall stretching can increase myometrial oxytocin receptor expression and in turn increase oxytocin-induced uterine contractions during normal labor [13]. Other studies have suggested that during the final stages of pregnancy, which are close to parturition, threshold increases in uterine stretching cause the upregulation of contraction-associated proteins (CAPs) that can also play a role in the induction of labor [14, 15].

Potassium channel TREK-1 is a type of CAP, and have been found to be expressed in the human and rat myometrium, particularly during pregnancy [16]. Based on the topology of the K+ channel subunits, these TREK K2P channels are thought to maintain background outward K+ current and resting membrane potential and counterbalance membrane depolarization and muscle contractions during pregnancy [17].

The activity of the TREK-1 channel has been shown to be regulated by numerous factors, including arachidonic acid (AA), L-methionine (L-M), pH, stretching, phosphorylation, nitric oxide, and temperature [18]. The present study indicates that the TREK-1 channel activator AA causes a dramatic enhancement in myometrial contractility, while its inhibitor L-M does not significantly increase contractility.
Our study demonstrated that prolonged stretch treatment decreased the myometrial contractility of the late-late pregnant rat uterus, which was similar to its myometrial relaxation in the early stage of pregnancy. It is unclear whether the pregnancy-associated and stretching-related reductions in the uterine contractions observed in late-late pregnancy, which is very close to parturition, reflect changes in TREK-1 expression/activity.

To determine whether prolonged stretching inhibits the myometrial contractility of the late-late pregnant rat uterus via the TREK-1 channel, isometric contraction measurements were performed to demonstrate that the reduced myometrial contractions caused by prolonged stretching were reversed under treatment with the TREK-1 inhibitor L-M, while there was no further contraction decrease under treatment with the TREK-1 activator AA.

The changes in the myometrial contractility of late-late pregnant uterus treated with L-M or AA after prolonged stretching suggest that the TREK-1 channel plays a vital role in the process of stretching-mediated regulation of uterine contractions before labor. The reversed contraction caused by L-M suggests that prolonged stretching decreases uterine contractions by regulating TREK-1 activity, while the myometrial relaxation caused by AA reaches saturation, possibly due to functional limitations in the TREK-1 channel.

**Conclusion**

A certain degree of stretching can maintain the balance of myometrial contraction and relaxation in late-late pregnant rats. The expression dynamics of potassium channel TREK-1 may be key to maintaining myometrial contraction/relaxation alterations. The limitation of the present study is that all of the data were collected from rats, which may differ from human beings in terms of these effects.

**Abbreviations**

potassium chloride, KCl

carbon dioxide, CO₂

area under the curve, AUC

arachidonic acid, AA

L-methionine, L-M

**Declarations**

Ethics approval and consent to participate
All procedures were performed in accordance with the guidelines of the Institutional Animal Care and Use Ethics Committee at the First Affiliated Hospital of Anhui Medical University, Hefei, China.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was funded by University Natural Science Foundation of Anhui Educational Committee (KJ2018A0188) to Zongzhi Yin and partly supported by a grant from National Natural Science Foundation of China (NO.81300514) to Zongzhi Yin.

Authors’ contributions

Conception and planning: Cao Y, Yin Z, Li Y.

Carrying out by Yin Z, Li Y, Li D, Su J, Yang Y, Shen B.

Analysing and writing the manuscript: Yin Z, Li Y, Cao Y.

Acknowledgements

We thank Dr. Raouf A. Khalil from Vascular Surgery, Research laboratories, Division of Vascular and Endovascular Surgery, Brigham and Women's Hospital, and Harvard Medical School to help the research design.

References

1. Smith R. Parturition. N Engl J Med. 2007; 356:271-83. http:// doi: 10.1056/NEJMra061360.

2. Renthal NE, Williams KC, Mendelson CR. MicroRNAs–mediators of myometrial contractility during pregnancy and labour. Nat Rev Endocrinol. 2013 Jul;9(7):391-401. http:// doi: 10.1038/nrendo.2013.96.

3. Smith R, Imtiaz M, Banney D, et al. Why the heart is like an orchestra and the uterus is like a soccer crowd. Am J Obstet Gynecol. 2015; 213:181-5. http:// doi: 10.1016/j.ajog.2015.09.105.
4. Yin ZZ, Sada AA, Reslan OM, et al. Increased MMPs expression and decreased contraction in the rat myometrium during pregnancy and in response to prolonged stretch and sex hormones. Am J Physiol, 2012, 303(1):E55-70. http://doi:10.1152/ajpendo.00553.2011.

5. Yin ZZ, He W, Li Y, et al. Adaptive reduction of human myometrium contractile activity in response to prolonged uterine stretch during term and twin pregnancy. Role of TREK-1 channel. Biochem Pharmacol. 2018 Jun;152:252-263. http://doi: 10.1016/j.bcp.2018.03.021.

6. Noble K, Matthew A, Burdyga T, et al. A review of recent insights into the role of the sarcoplasmic reticulum and Ca entry in uterine smooth muscle. Eur J Obstet Gynecol Reprod Biol, 2009, 144 Suppl 1: S11-19. http://doi: 10.1016/j.ejogrb.2009.02.010.

7. Brainard AM, Korovkina VP, England SK. Potassium channels and uterine function. Semin Cell Dev Biol, 2007 Jun;18(3):332-339. http://2007 Jun;18(3):332-9.

8. Li Q, Sun M. Effects of potassium ion channels in term pregnant myometrium. J Obstet Gynaecol Res, 2012, 38(2): 479. http://doi: 10.1111/j.1447-0756.2011.01711.x.

9. Tichenor JN, Hansen ET, Buxton IL. Expression of Stretch-activated Potassium Channels in Human Myometrium. Proc West Pharmacol Soc, 2005;48:44-8. http://doi: 10.1371/journal.pone.0012372.

10. Monaghan K, Baker SA, Dwyer L, et al. The stretch-dependent potassium channel TREK-1 and its function in murine myometrium. J Physiol, 2011, 589(5):1221-1233. http://doi: 10.1113/jphysiol.2010.203869.

11. Yin Z, Li Y, He W, et al. Progesterone inhibits contraction and increases TREK-1 potassium channel expression in late pregnant rat uterus. Oncotarget. 2018 9(1):651-661 http://doi: 10.18632/oncotarget.23084.

12. Parkington HC, Stevenson J, Tonta MA, et al. Diminished hERG K+ channel activity facilitates strong human labour contractions but is dysregulated in obese women. Nat Commun, 2014, 5: 4108. http://doi: 10.1038/ncomms5108.

13. Terzidou V, Sooranna SR, Kim LU, et al. Mechanical stretch up-regulates the human oxytocin receptor in primary human uterine myocytes. J Clin Endocrinol Metab 2005;90:237-46. http://doi: 10.1210/jc.2004-0277.

14. Mohan AR, Sooranna SR, Lindstrom TM, et al. The effect of mechanical stretch on cyclooxygenase type 2 expression and activator protein-1 and nuclear factor-kappaB activity in human amnion cells. Endocrinology 2007;148:1850-7. http://doi: 10.1210/en.2006-1289.

15. Li Y, Reznichenko M, Tribe RM, et al. Stretch activates human myometrium via ERK, caldesmon and focal adhesion signaling. PloS one 2009;4:e7489. http://doi: 10.1371/journal.pone.0007489.

16. Buxton IL, Singer CA, Tichenor JN. Expression of stretch-activated two-pore potassium channels in human myometrium in pregnancy and labor. Biol Reprod. 2015 Nov;93(5):122. doi: 10.1095/biolreprod.115.129791

17. Heyman NS, Cowles CL, Barnett SD, et al. TREK-1 currents in smooth muscle cells from pregnant human myometrium. Am J Physiol Cell Physiol. 2013; 305:C632-42. doi: 10.1152/ajpcell.00324. http://doi: 10.1152/ajpcell.00324.2012.
Dedman A, Sharif-Naeini R, Folgering JH, et al. The mechano-gated K(2P) channel TREK-1. Eur Biophys J, 2009, 38(3): 293-303. http://doi: 10.1007/s00249-008-0318-8.

Figures

Figure 1

Effects of prolonged stretching on oxytocin-induced contractions. In isolated late-late pregnant rat uterine strips, oxytocin caused a concentration-dependent increase in the maintained contraction, reaching a peak at 10−7 M in the rat uterine strips, as previously described. The AUC/g tissue of the oxytocin-induced contraction was calculated for 20, 40, and 60 min with prolonged stretching (8 g) or basal stretching (2 g) treatment. The data are presented as the mean ± SEM; 2 g, n =10; 8 g, n=7. *Significantly different (P <0.05)
Figure 2

Effect of TREK-1 activation on late-late uterine contractions. Uterine strips from late-late pregnancy were either not treated (2 g stretch) or pretreated with TREK-1 activator arachidonic acid (10-5 M) for 1 h of prolonged stretching (8 g) or 8 g + arachidonic acid for 18 h and then stimulated with oxytocin (10–7 M) for 1 h (A). The AUC/g tissue for oxytocin-induced contractions was calculated for 20, 40, and 60 min (B,C,D,E). Data are presented as the means ± SEMs, 2 g: n=10, 2 g+AA: n=10, 8 g: n=7, 8 g+AA: n=9. * # & Significantly different (P < 0.05)
Figure 3

Effect of inhibition TREK-1 on late-late uterine contractions Uterine strips from late-late pregnancy were either not treated (2 g stretch) or pretreated with TREK-1 inhibitor L-methionine (1 mM) for 1 h of prolonged stretching (8 g) or 8 g + L-methionine for 18 h and then stimulated with oxytocin (10−7 M) for 1 h (A). The oxytocin-induced contraction in AUC/g tissue was calculated for 20, 40, and 60 min (B,C,D,E). Data are presented as the means ± SEMs. 2 g: n=10, 2 g+L-M: n=10, 8 g: n=7, 8 g+L-M: n=9. * # & Significantly different (P < 0.05)