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Differential regulation by mechanical stretch of the expressions of large-conductance Ca^{2+}-activated K^+ channel and L-type voltage-dependent Ca^{2+} channel in rat uterine smooth muscle cells

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Running title: The expressions of BK_{Ca} channel and L-VDCC in USMCs under stretch

Abstract

Large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channel and L-type voltage-dependent Ca^{2+} channel (L-VDCC) play important roles in regulating uterine contractility. The uterus stretch, occurring during pregnancy, is a critical factor to trigger uterine contraction. However, how mechanical stimuli impact the two channels remains unknown. Here we investigated the effects of exposure to mechanical stretches with varying magnitudes and durations on expressions of the two channels in rat uterine smooth muscle cells (USMCs). Our results show that stretch downregulates the BK_{Ca} channel expression but upregulates the L-VDCC expression. These findings are helpful to better understand the roles of L-VDCC and BK_{Ca} channel in stretch-triggered uterine contraction.

Key words: BK_{Ca} channel, L-VDCC, stretch, uterine smooth muscle cell

Introduction

The uterus stretch, occurring during pregnancy, is a critical factor to trigger uterine contraction (Kasai, 1995). Therefore, the uterus needs to keep quiescence to permit fetus development through complex mechanisms, overcoming the tendency of the uterine contraction. L-type voltage-dependent Ca^{2+} channel (L-VDCC) and large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channel are important mechanisms involved in these processes (Carvajal and Weiner, 2003). L-VDCC is a multimeric complex consisting of a pore-forming α1 subunit and regulatory β, γ and α_2/δ subunits, with the α1 subunit determining its functional characterization (Catterall, 1991). BK_{Ca} channel is composed of four pore-forming α-subunits and accessory tissue-specific β-subunit (β1-4), with β1 being mainly expressed in smooth muscles. L-VDCC primarily mediates Ca^{2+} influx in uterus and consequent induction of uterine contraction and, by contrast, BK_{Ca} channel is activated by intracellular Ca^{2+} and its activation causes membrane hyperpolarization, thereby closing L-VDCC and leading to uterine quiescence (Brainard et al., 2007). Increasing research attempts to understand the two channels for their roles in determining how the uterus maintains quiescent during pregnancy and become contractile at labor. Hormone regulation and inflammation are well-defined factors to trigger uterine contraction, and they are interacted with stretch in synergic or differential manner to regulate the contraction-associated events in uterus (Ou, 1998; Ou, 1997; Sooranna, 2004). Here we examine the hypothesis that stretch impacts the expressions of BK_{Ca} channel and L-VDCC by exposing rat USMCs to stretches of magnitudes and durations that are similar to those occurring in different stages of gestation. Our results show that stretch downregulates the BK_{Ca} channel expression but upregulates the L-VDCC expression. These findings are helpful to better understand the roles of L-VDCC and BK_{Ca} channel in stretch-triggered uterine

contraction.

Materials and Methods

Cell isolation and culture

All experiments were approved by the Animal Research Ethics Committee of Beihang University. Isolation of USMCs from female Sprague–Dawley rats (~200 g weight) and culture were described in the Supplementary File. BMSCs were characterized by immunofluorescence staining for smooth muscle cell-specific α-actin (α-SMA) (see Fig. S1). Cells of passage 3-5 were used.

Mechanical stretch

Cells were seeded at a density of 1×10^5 on silicone chambers pre-coated with 50 μg/ml collagen I (Becton Dickinson). When cells reached 80% confluence, they were exposed to stretch by 5%, 10% and 20% for 24, 36 and 48 h, respectively, using the stretch device (STREX, Japan). Cells without stretch were used as static control (SC).

RNA *isolation* and reverse transcription-quantitative PCR (RT-qPCR)

Total RNA extraction and RT-qPCR performance were described in the Supplementary File. The primers used were listed in Table S1. GAPDH served as the internal control. Results were calculated using the $2^{-\Delta\Delta CT}$ method.

Protein expression determination

Protein expression was determined by flow cytometry as previously described (Jia et al., 2013). Briefly, cells were fixed, permeabilized, and incubated with primary antibodies against α- and β1-subunits of the

BK_{Ca} channel or α1c subunit of L-VDCC (Alomone), respectively. After that, cells were stained with fluorescein isothiocyanate (FITC)-conjugated secondary antibodoes (Sigma). Cells were incubated with isotype control IgG (R&D Research) as a negative control (NC). The fluorescence intensity was determined by FACSCalibur (Becton Dickinson) and analysed using CellQuest software. The specific fluorescence intensity for each case was derived by subtracting the fluorescence of NC cells from the total intensity.

Statistical analysis

Data are presented as mean ± standard deviation, where appropriate. Statistical analyses were conducted using Student's t-test or one-way ANOVA and *post hoc* Fisher's test as indicated. $P < 0.05$ was considered statistically significant.

Results

Stretches down-regulate BK_{Ca} channel expression

We first examined the effect of stretches on the expression of BK_{Ca} channel α-subunit (Fig.1A-C). Fig.1 A show that the mRNA level in cells, after exposed to stretches (5%-20%) for 24-48 h was reduced compared to that in SC cells. Similarly, the protein expression level in stretched cells was less than that in SC cells (Fig.1B-C). We also examined the effect of exposure to 5-20% stretches for 24-48 h on the expression of BK_{Ca} channel β1-subunit (Fig.1D-F). Compared to that in SC cells, both the mRNA expression (Fig. 2D) and protein expression levels were decreased (Fig. 2E-F). While the expression of both α- and β1-subunit was overall dependent of stretch magnitude and stretch duration, the dependence appeared relatively more noticeable for the β1-subunit.

Stretches up-regulate L-VDCC expression

Considering the critical role of the L-VDCC in regulating the contractility of uterus as mentioned in introduction, we were also interested in the effects of exposure to stretches (5-20%) for 24-48 h on the L-VDCC α1c subunit expression (Fig. 2). The mRNA expression level in stretched cells was significantly elevated compared with that in SC cells (Fig. 2A). Consistently, the protein expression level in stretched cells was much higher than that in SC cells (Fig. 2B-C). Overall, these results show that the expression of the α1c-subunit was significantly upregulated by stretch in a dose- and duration-dependent manner.

Discussion

There are few studies on stretch regulation of the BK_{Ca} channel although it is abundantly expressed in the uterus (Brainard et al., 2007). We used stretches with varying magnitudes and durations to mimic the uterus extensions that occur in different gestational stages. Our results show that exposure to stretches downregulates the expression of BK_{Ca} channel, both the α-subunit and β1-subunit (Fig. 1). From this standpoint, such stretch-induced decrease in the expression of BK_{Ca} channel observed in our study is in agreement with the results reported by other groups that the expression of BK_{Ca} channel is diminished during late pregnancy and at labor compared with non- or early pregnancy (Gao et al., 2009; Matharoo-Ball et al., 2003). In addition, we found that the expression of β1-subunit declined more drastically than that of α-subunit in response to stretch. This result is consistent with our previous finding showing that alteration of the β1-subunit expression in vascular smooth muscle cells is more drastic than that of α-subunit after exposure to shear stress (Jia et al., 2013). Taken together, our finding suggests that alteration in the β1-subunit plays a more important role in tuning muscle mechanical activity (Tanaka et al., 2004). Similar to the BK_{Ca} channel, the L-VDCC changes its expression during gestational stage, which is known to elevate towards labor (Collins et al., 2000; Mershon et al., 1994). Consistently, our results show a stretch-induced increase in the expression of L-VDCC (Fig. 2). The L-VDCC has been suggested to mainly mediate stretch-induced rise in intracellular Ca^{2+} concentration (Kasai, 1995). Our finding raises a possibility that stretch-induced increase in its expression may contribute such L-VDCC-mediated Ca^{2+} rise. Collectively, our study reveals that, stretches exert differential regulation of the expressions of BK_{Ca} channel and L-VDCC. This finding would be helpful for understanding the roles of the two channels in stretch-triggered uterine contraction. Further

research is necessary to investigate the integrated effects of stretch, hormones and inflammation on the two channels.

Conflict of interest

The authors confirm that there is no conflict of interest.

Acknowledgments

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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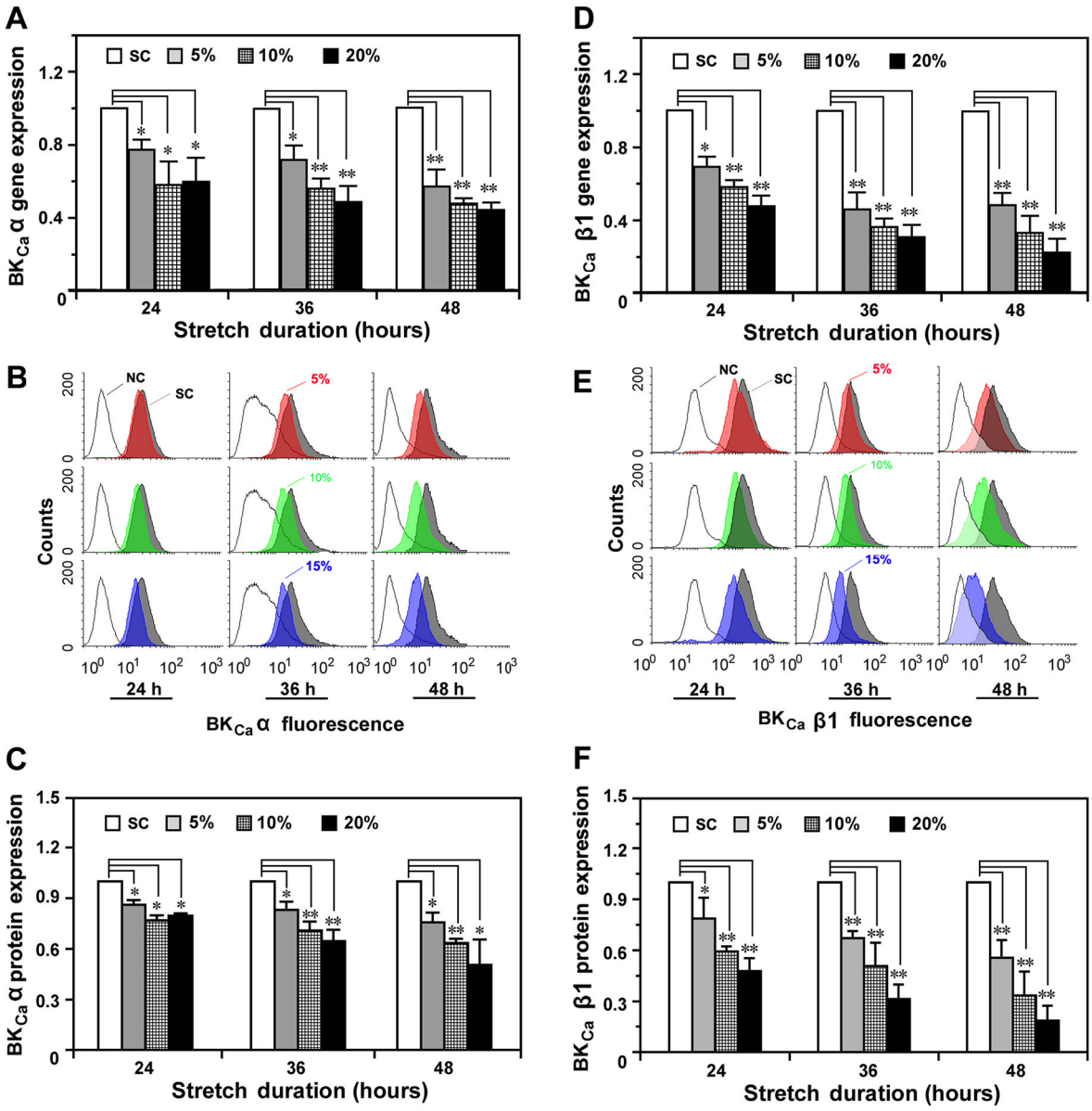
Figure captions

Figure 1 Stretch decreases the expression of α- and β1-subunit of BK_{Ca} channel in USMCs. A&D Mean values of BK_{Ca} α- and β1-sunbuit expression on gene level were determined by RT-qPCR. B&C Protein expression level was determined by flow cytometry. Representative graphs of BK_{Ca} α-sunbunit fluorescence (B); Mean value of BK_{Ca} α-subunit protein expression (normalized to control) (C). E&F Protein expression level was determined by flow cytometry. Representative graphs of BK_{Ca} β1-sunbuit fluorescence (E); Mean value of BK_{Ca} β1-sunbuit protein expression (normalized to control) (F). $*P < 0.05$ and $*P < 0.001$ vs. control (n=3)

Figure 2 Stretch increases the expression of LVDCC α1c subunit in USMCs. A Mean value of LVDCC α1c-subunit gene expression was determined by RT-qPCR. B&C Protein expression level was determined by flow cytometry. Representative graphs of LVDCC α1c-subunit fluorescence (B); Mean value of LVDCC α1c subunit protein expression (normalized to control). (C). $*P < 0.05$ and $*P < 0.001$ vs. control (n=3)

Revised figure 1



Revised figure 2