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Increase of L-type Ca^{2+} current by protease-activated receptor 2 activation contributes to augmentation of spontaneous uterine contractility in pregnant rats

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ABSTRACT

We evaluated the effects of protease-activated receptor (PAR)-2 on spontaneous myometrial contraction (SMC) in isolated term pregnant myometrial strips of rat, and elucidated the cellular mechanisms of this effect using a conventional voltage-clamp method. In isometric tension measurements, trypsin and SL-NH_2, PAR-2 agonists, significantly augmented SMC in frequency and amplitude; however, boiled trypsin (BT) and LR-NH_2 had no effect on SMC. These stimulatory effects of PAR-2 agonists on SMC were nearly completely occluded by pre-application of Bay K 8644, an L-type voltage-gated Ca^{2+} channel activator, thus showing the involvement of L-type voltage-gated Ca^{2+} channels in PAR-2-induced augmentation of SMC. In addition, PAR-2 agonists significantly enhanced L-type voltage-gated Ca^{2+} currents ($I_{Ca\text{-}L}$), as measured by a conventional voltage-clamp method, and this increase was primarily mediated by activation of phospholipase C (PLC) and protein kinase C (PKC) via G-protein activation. Taken together, we have demonstrated that PAR-2 may actively regulate SMC during pregnancy by modulating Ca^{2+} influx through L-type voltage-gated Ca^{2+} channels, and that this increase of $I_{Ca\text{-}L}$ may be primarily mediated by PLC and PKC activation. These results suggest a cellular mechanism for the pathophysiological effects of PAR-2 activation on myometrial contractility during pregnancy and provide basic and theoretical information about developing new agents for the treatment of premature labor and other obstetric complications.

1. Introduction

Proteases such as thrombin and trypsin strongly stimulate contractions of uterine myometrium, causing preterm labor [1–3]. In addition, urinary trypsin inhibitor suppresses spontaneous myometrial contraction (SMC) to prevent preterm deliveries in pregnant mice and humans [4,5], suggesting the involvement of trypsin in regulating uterine contractility and preterm labor. Intensive research has been performed to reveal a cause of protease-induced myometrial contraction; however, a detailed mechanism remains to be elucidated.

Protease-activated receptors (PARs) are G-protein coupled receptors (GPCRs) with seven transmembrane domains. To date, four types of PARs (PAR 1–4) have been identified. These proteases activate PARs by cleaving the amino terminal sequence of the extracellular N-terminal domains. Cleavage at these specific sites by proteases exposes the new N-terminal as a "tethered ligand" and binds to a site in the second extracellular loop to initiate G-protein coupling and intracellular cell signaling. Recently, it was reported that PAR-2, which is a specific receptor for trypsin, exists in pregnant rat uterine tissue [6,7] and that this PAR-2 is actively involved in trypsin-evoked myometrial contraction in pregnant rats [7,8]. This effect is independent of mast cell activation or cyclo-oxygenase pathway products, suggesting that PAR-2 located in the myometrium, not in the mast cell, may play an important role in regulating SMC by PAR-2 activation. However, a detailed mechanism has not yet been elucidated.

Contractions in pregnant myometrium are usually elicited by action potentials superimposed onto slow waves. Regardless of the causes that evoke the firing of action potentials in uterine smooth muscle, their results converge on depolarization and opening of voltage-gated L-type Ca^{2+} channels, and Ca^{2+} influx into the cell through the open channels leads to myometrial contraction [9–11]. For example, blockers of voltage-gated L-type Ca^{2+} channels such as nifedipine, have been reported to effectively abolish uterine spontaneous contraction in pregnant myometrium in vitro [12–14]. In addition, Bay K 8644, a voltage-dependent Ca^{2+} channel activator, has been shown to effectively potentiate or even initiate SMC [15,16]. So, any agent that can modulate voltage-gated L-type Ca^{2+} channels may regulate SMC.

Taken together, it is possible that trypsin-induced augmentation of SMC may be mediated through an increase in voltage-gated

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L-type Ca^{2+} current (I_{Ca-L}) by PAR-2 activation. To test this hypothesis, we determined the effects of PAR-2 agonists (trypsin and SL-NH_2, a PAR-2 activating peptide) as a stimulant of voltage-gated L-type Ca^{2+} channels by comparing the effects of PAR-2 activation on SMC with those of Bay K 8644 in isolated, longitudinal myometrial strips of pregnant rat using isometric tension measurement. We confirmed the effects of PAR-2 on voltage-gated L-type Ca^{2+} channels by measuring I_{Ca-L} using a conventional voltage-clamping method. We demonstrate that PAR-2 activation effectively augmented SMC by augmentation of I_{Ca-L} in pregnant rat.

2. Materials and methods

This study was conducted in accordance with the National Institute of Health (NIH) Guide for the Care and Use of Laboratory Animals. All procedures were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee.

2.1. Measurement of isometric tension in uterine myometrial strips

Myometrial strips were prepared from Sprague–Dawley rats in late pregnancy (18–20 days gestation), as described previously [17]. Briefly, we removed the blood from a blood vessel by perfusing the rats transcardially with cold Dulbecco's phosphate buffer saline (D-PBS) for 15 min under enflurane anesthesia (3%, 2–3 min) to prevent PAR-2 activation by coagulation factor VIIa or Xa previously. Longitudinal myometrial strips, approximately 1.5 × 3 mm from each horn, were dissected out with fine scissors under a binocular microscope. Both ends of the tissue strip were tied with thin threads. One end was connected to a force–displacement transducer to monitor muscle tension and the other end was attached to a tissue holder. The PSS solution was maintained at 37 °C and was continuously aerated with 100% O_2. Muscle strips were stretched passively to an optimal length by imposing a stretch of 140% of resting length and equilibrated for 60 min.

2.2. Longitudinal uterine myocyte isolation

The longitudinal muscle layer of myometrium was cut into small pieces and placed in Ca^{2+}-free PSS. The Ca^{2+}-free PSS was then replaced with PSS containing 30 μM Ca^{2+} (low Ca^{2+} PSS), and 1 h incubations at 37 °C were carried out in fresh, low-Ca^{2+} PSS containing collagenase (0.3 mg/ml), papain (0.6 mg/ml), and bovine serum albumin (1 mg/ml). After enzyme digestion, tissue fragments were suspended in a fresh 120 μM Ca^{2+} containing PSS and gently agitated. The resulting suspension was centrifuged at 800*g* for 2 min, and the cells were resuspended in a 0.5 mM Ca^{2+} containing PSS, aliquoted into 12 mm poly-L-lysine-coated cover glasses, and stored in a humidified atmosphere at 4 °C. Experiments were carried out within 12 h of harvesting at (22–24 °C).

2.3. Electrophysiology

I_{Ca-L} was recorded using conventional whole-cell techniques. Electrode resistance varied from 3 to 5 MΩ when cells were filled with internal solution. We performed measurements using an Axopatch 1D patch-clamp amplifier (Molecular Devices, Sunnyvale, CA, USA). Voltage and current commands and digitization of membrane voltages and currents were controlled using a Digidata 1322 A interfaced with Clampex 9.2 (Molecular Devices, Sunnyvale, CA, USA) on an IBM-compatible computer. We analyzed data using Clampfit 9.2 (Molecular Devices, Sunnyvale, CA, USA) and Prism 4.0 (GraphPad, San Diego, CA, USA). Currents were low-pass filtered at 2 kHz using a four-pole Bessel filter in the amplifier. Capacitance (Cm) values were taken from automatically calculated recordings by the pClamp 9.2 software. Multiple independently-controlled syringes served as reservoirs for a gravity-driven fast drug perfusion system. Switching between solutions was accomplished by manually controlled valves. All experiments were conducted at room temperature.

2.4. Solutions and drugs

The PSS contained the following (in mM): 140 NaCl, 5.4 KCl; 1.2 $MgCl_2$, 10 HEPES, 2.5 $CaCl_2$, 12 Glucose; the solution was adjusted to pH 7.4 with NaOH. Ca^{2+}-free PSS was made by simply omitting Ca^{2+} from normal PSS. Low Ca^{2+} PSS was made by adding Ca^{2+} (30 or 120 μM) to Ca^{2+}-free PSS. The internal (pipette) solution contained the following (in mM): 140 $CsCl_2$, 1.2 $MgCl_2$, 4 MgATP, 0.4 Na_2GTP, 10 phosphocreatine, 10 HEPES, and 10 EGTA; the solution was adjusted to pH 7.2 with CsOH. The external (bath) solution contained 155 tetraethylammonium (TEA)–Cl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 14 glucose, and 10.5 HEPES; the solution was adjusted to pH 7.4 with TEA–OH. The D-PBS solution with glucose contained the following (in mM): 2.67 KCl, 1.47 KH_2PO_4, 137.93 NaCl, 8.06 Na_2HP_4–$7H_2O$, 5.56 D-glucose; the solution was adjusted to pH 7.4 with HCl. The phosphate-buffered saline (PBS) contained the following (in mM): 137 NaCl, 2.7 KCl, 10.14 Na_2HPO4, 1.76 KH_2PO4, 0.05% Tween 20. Bay K 8644 was purchased from Tocris (Tocris Cookson Inc., Bristol, UK). SL-NH_2 and LR-NH_2 were purchased from COSMO (COSMO Genetech Inc., Seoul, Korea). All other drugs were purchased from Sigma–Aldrich Chemicals. Bay K 8644 and calphostin C were dissolved in dimethyl sulfoxide (DMSO). All other drugs were dissolved in distilled water as stock solutions (1–100 mM).

2.5. Data analysis

Data are presented as mean ± SEM, with the number of experiments indicated in parentheses. The frequency of SMC was presented as the number of spontaneous contractions per 5 min. The amplitude of SMC was presented in grams (g). The concentration–response curves for the PAR-2 agonists in myometrial contraction were calculated by fitting the data to a single-site binding isotherm using least-squares nonlinear regression, and two curve fits were compared by the *F*-test using Prism 4.0 (GraphPad). We used Student's unpaired *t*-tests to compare the means of two different groups; however, we used a paired *t*-test to compare the effects of PAR-2 agonists before and after application. Differences were considered to be significant at $P < 0.05$.

3. Results

3.1. Effects of PAR-2 agonists on SMC

First, we confirmed the stimulatory effect of PAR-2 on SMC in pregnant rat. Consistent with previous data [7,8], trypsin (30 nM) augmented SMC significantly, by 62.2 ± 5.2% in frequency (control, 5.8 ± 0.9, $n = 6$; trypsin, 8.4 ± 1.0, $n = 6$; $P < 0.05$) and 28.4 ± 5.8% in amplitude (control, 1.1 ± 0.1, $n = 6$; trypsin, 1.4 ± 0.2, $n = 6$; $P < 0.05$). However, boiled trypsin (BT) (30 nM) did not have any effect on the frequency (control, 3.2 ± 0.5, $n = 6$; BT, 3.0 ± 0.4, $n = 6$; $P > 0.05$) or amplitude (control, 0.7 ± 0.1, $n = 6$; BT, 0.7 ± 0.1, $n = 6$, $P > 0.05$) of SMC (Fig. 1A and B). Likewise 100 μM SL-NH_2 also enhanced SMC by 64.7 ± 8.9% in frequency (control, 4.0 ± 0.5, $n = 6$; SL-NH_2, 7.3 ± 1.1, $n = 6$, $P < 0.05$) and 27 ± 4.0% in amplitude (control, 0.8 ± 0.1, $n = 6$; SL-NH_2, 1.0 ± 0.2, $n = 6$, $P < 0.05$); however, 100 μM LR-NH_2, a control reverse peptide, had no influence on frequency (control, 4.2 ± 1.0, $n = 6$; LR-NH_2, 4.0 ± 0.9, $n = 6$, $P > 0.05$) or amplitude (control, 0.6 ± 0.1, $n = 6$; LR-NH_2, 0.6 ± 0.1, $n = 6$, $P > 0.05$)

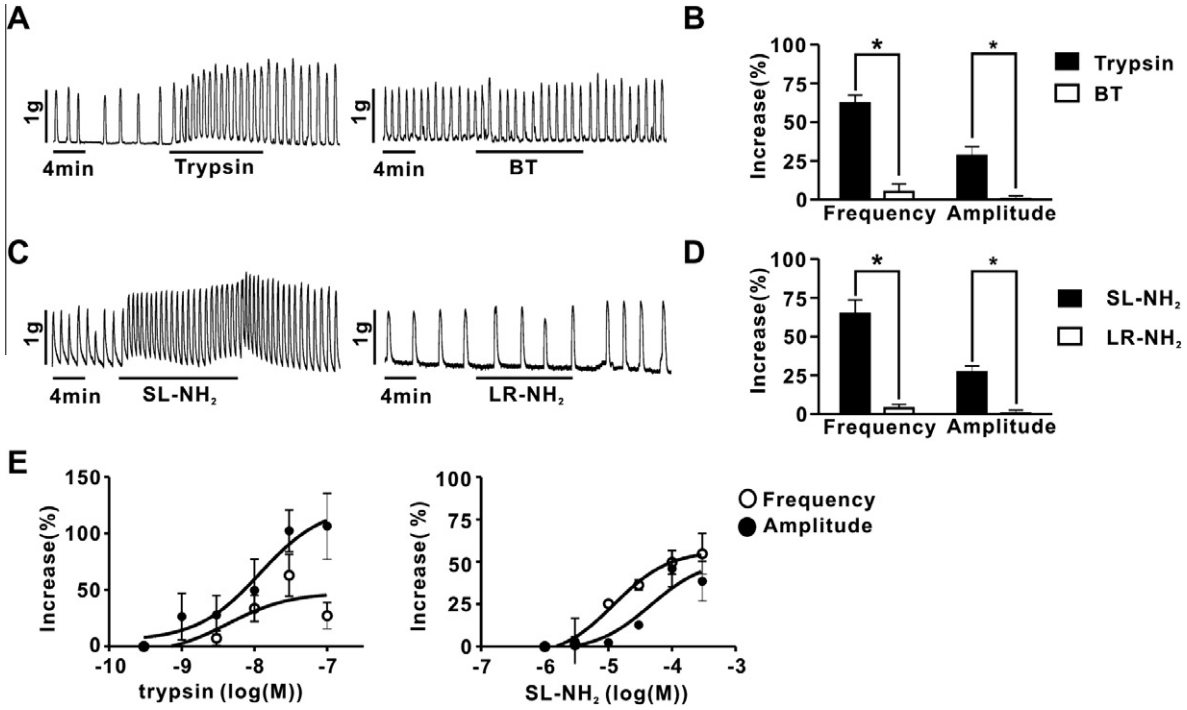

Fig. 1. Effects of PAR2 agonist on spontaneous contraction in pregnant rat myometrium. (A) Representative trace of effect of 30 nM trypsin (left) or 30 nM BT (right) on SMC in pregnant rat tissue. (B) Effects of trypsin and BT on mean frequency and amplitude of SMC. (C) Representative trace effect of 100 μM $SL\text{-}NH_2$ (left) or 100 μM $LR\text{-}NH_2$ (right) on SMC in pregnant rat tissue. (D) Effects of $SL\text{-}NH_2$ and $LR\text{-}NH_2$ on mean frequency and amplitude of SMC. (E) Concentration response curve for PAR-2 agonists. The changes in frequency and amplitude of SMC are expressed relative to control. $n = 5$ in all groups.

(Fig. 1C and D). To determine the concentration-response relationships of the PAR-2 agonist effects on SMC, trypsin and $SL\text{-}NH_2$ were applied to the myometrial strips of pregnant rat externally and cumulatively at ascending concentrations of 0.3–100 nM and 1–300 μM, respectively. As shown in Fig. 1E, the degree of augmentation was estimated as the ratio of the increase in frequency or amplitude of SMC to the control. The EC_{50} of trypsin (mean values) were 4.3 ± 3.5 nM in frequency and 11.9 ± 5.5 nM in amplitude. In addition, the EC_{50} of $SL\text{-}NH_2$ (mean values) were 12.3 ± 4.4 nM in frequency and 4.6 ± 7.1 nM in amplitude. The concentrations at which trypsin and $SL\text{-}NH_2$ augmented SMC sub-maximally were about 30 nM and 100 μM, respectively. These concentrations, therefore, were used in all subsequent experiments.

3.2. Effects of Bay K 8644 on stimulatory effect of PAR2 on SMC in pregnant rat

To determine the possibility that PAR-2 agonists may augment SMC in pregnant rat by modulating L-type voltage-gated Ca^{2+} channels, we tested whether Bay K 8644, a Ca^{2+} channel activator, can influence the stimulatory effect of PAR-2 on SMC in pregnant rat.

Consistent with previous reports,[15,16] Bay K 8644 (1 μM) enhanced SMC by 45 ± 10.8% in frequency (control, 3.0 ± 0.7; Bay K 8644, 4.3 ± 0.9; $n = 4$; $P < 0.05$) and 118.8 ± 38.3% in amplitude (control, 0.9 ± 0.3; Bay K 8644, 1.5 ± 0.6; $n = 4$; $P < 0.05$) (Fig. 2A). However, subsequent application of trypsin (30 nM) in the presence of Bay K 8644 hardly augmented SMC by 5 ± 0.5% in frequency (Bay K 8644 + Trypsin, 4.5 ± 1.0; $n = 4$; $P > 0.05$) and 2.1 ± 0.3% in amplitude (Bay K 8644 + Trypsin, 1.5 ± 0.4; $n = 4$; $P > 0.05$, Bay K 8644 versus Bay K 8644 + Trypsin; one-way ANOVA, Tukey's test) (Fig. 2A and B). Likewise, Bay K 8644 enhanced SMC by 71.7 ± 22.9% in frequency (control, 3.0 ± 0.8; Bay K 8644, 4.3 ± 0.6; $n = 4$; $P < 0.05$) and 56.3 ± 6.3% in amplitude (control, 1.5 ± 0.4; Bay K 8644, 2.6 ± 0.6; $n = 4$; $P < 0.05$) (Fig. 2C and D). Bay K 8644 nearly completely occluded the stimulatory effect of $SL\text{-}NH_2$ (100 μM) on SMC by 0.6 ± 0.4% in frequency (Bay K 8644 + $SL\text{-}NH_2$, 4.5 ± 1.0; $n = 4$; $P < 0.05$) and 0.9 ± 0.6% in amplitude (Bay K 8644 + Trypsin, 2.7 ± 0.5; $n = 4$; $P < 0.05$) (Fig. 2C and D). These data suggest that the stimulatory effect of PAR-2 on SMC in pregnant rat may be mediated by modulating L-type voltage-gated Ca^{2+} channels.

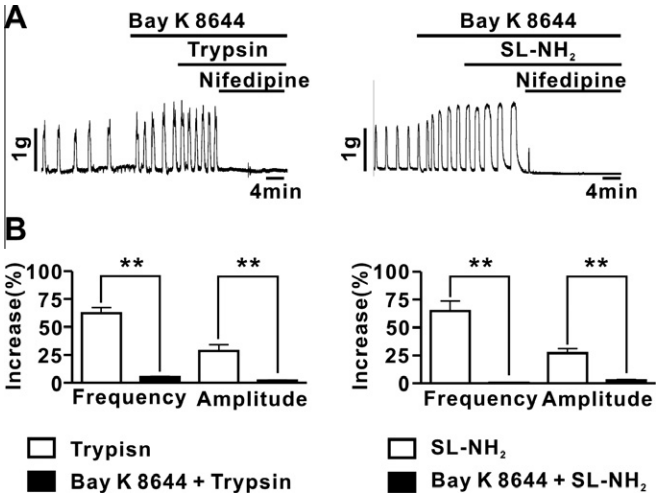

Fig. 2. Effects of Bay K 8644 on stimulatory effect of PAR-2 activation on SMC in pregnant rat tissue. (A) Representative trace of cumulative effects of trypsin (30 nM) (left) or SL-NH2 (100 μM) (right) in the presence of Bay K 8644 (1 μM). Nifedipine (10 μM) completely abolished augmented SMC by PAR-2 agonists. (B) Effects of Bay K 8644 on stimulatory effect of trypsin (30 nM) (left) or SL-NH2 (100 μM) (right) on mean frequency and amplitude of SMC. The asterisk symbol (**) indicates that the mean value is significantly different from the respective control ($n = 4$, $P < 0.01$).

3.3. Effect of PAR-2 agonist on $I_{Ca\text{-}L}$ in rat myometrial cells

To determine the effect of PAR-2 on L-type voltage-gated Ca^{2+} channels directly, $I_{Ca\text{-}L}$ was measured using a conventional voltage-clamp method. $I_{Ca\text{-}L}$ was evoked by 200 ms depolarizing step pulses to a test potential of 0 mV from a holding potential of −60 mV. Fig. 3 shows a typical example of $I_{Ca\text{-}L}$, just before and after application of the indicated PAR-2 agonists. Trypsin (30 nM) augmented $I_{Ca\text{-}L}$ by 25 ± 3% (control, 235.5 ± 23.1 pA; trypsin, 296.3 ± 24.3 pA, $n = 7$; $P < 0.05$) in an irreversible manner and the increased $I_{Ca\text{-}L}$ was maintained without significant change during the experiment (Fig. 3A, left and middle). Likewise, SL-NH_2 (100 μM) increased $I_{Ca\text{-}L}$ by 24 ± 3% (control, 233.9 ± 23.5 pA; SL-NH_2, 290.4 ± 25.4 pA, $n = 7$; $P < 0.05$) (Fig. 3B, left and middle). PAR-2 agonists increased $I_{Ca\text{-}L}$ over a potential range from −30 mV to + 40 mV according to the current–voltage (I–V) relationship (Fig. 3A and B, right); however, neither BT (30 nM) (control, 246.2 ± 24.9 pA; 258.3 ± 18.4 pA; $n = 6$; $P > 0.05$) nor LR-NH_2 (100 μM) (control, 225.1 ± 28.2 pA; LR-NH_2, 238.2 ± 26.3 pA; $n = 6$; $P > 0.05$) had any influence on $I_{Ca\text{-}L}$ (Supplementary Fig. 1).

3.4. Stimulatory effect of PAR-2 activation on I_{Ca} is GPCR dependent

We determined the involvement of G proteins in PAR-2 activation on $I_{Ca\text{-}L}$ using GDPβS, a hydrolysis-resistant GDP analogue known to prevent G protein activation [18]. As shown in Fig. 4A, intracellular dialysis of GDPbS (2 mM) prevented augmentation induced by trypsin (control, 25 ± 3%; trypsin, 4 ± 1.3%, $n = 6$ and 5, respectively) and SL-NH_2 (control, 24 ± 3%; SL-NH_2, 3 ± 0.9%, $n = 6$ and 5, respectively).

The cellular effect of PAR-2, a membrane of the Gq/11-coupled metabotropic family, is generally regarded to be mediated by the phospholipase C (PLC) signal pathway. We determined the involvement of PLC in PAR-2 activation on $I_{Ca\text{-}L}$ using neomycin, which is known to suppress PLC activation.[19] The presence of neomycin (10 μM) in the internal solution significantly prevented the $I_{Ca\text{-}L}$ augmentation produced by trypsin (control, 25 ± 3%; trypsin, 1.0 ± 0.7%, $n = 6$ and 5, respectively) and by SL-NH2 (control, 24 ± 3%; SL-NH_2, 1.0 ± 0.9%, $n = 6$ and 5, respectively) (Fig. 4B).

Finally, we examined whether protein kinase C (PKC), another cellular signal pathway of PLC, also contributed to the PAR-2 effect on I_{Ca}. As shown in Fig. 4C, Calphostin C (1 μM), a PKC antagonist, [20,21] in the internal solution significantly prevented the I_{Ca} potentiation produced by trypsin (control, 25 ± 3%; trypsin, 1.9 ± 1.2%, $n = 6$ and 6, respectively) and by SL-NH_2 (control, 24 ± 3.%; SL-NH_2, 1.9 ± 2.2, $n = 6$ and 6, respectively).

4. Discussion

Since PAR-2 has been suggested to be associated with trypsin-induced myometrial contraction [6,8], much attention has been paid to the physiological role of PAR-2 as a cause of preterm labor. However, controversies remain regarding the pathophysiological function of PAR-2 in pregnant myometrium. In fact, PAR-2 agonists potentiated SMC in term pregnant rats [8], suggesting a functional role of PAR-2 in regulating myometrial contractility. Freerksen et al. [7] reported that SL-NH_2 stimulated SMC in pregnant rats through other membrane receptors, such as histamine H1 receptor and serotonin receptor, as well as PAR-2. In the present study, trypsin and SL-NH_2 significantly augmented SMC in pregnant rat (Fig. 1); however, BT and LR-SH_2 had no effect, suggesting the involvement of PAR-2 in regulating SMC during pregnancy.

Although many mechanisms have been suggested to explain excitation-contraction coupling in pregnant myometrium [11,22,23], the most important factor in regulating myometrial contraction is an increase in intracellular Ca^{2+} concentrations [24,25], and a major source of Ca^{2+} is Ca^{2+} entry from the extracellular fluid through L-type voltage-gated Ca^{2+} channels [11]. So, any stimuli that affect the activity of L-type voltage-gated Ca^{2+} channels can modulate SMC. In fact, L-type voltage-gated Ca^{2+} channel antagonists, such as nifedipine, have been shown to abolish intracellular Ca^{2+} transients and SMC [12–14]. In addition, Bay K 8644, a dihydropyridine Ca^{2+} channel activator, has been shown to greatly augment SMC in pregnant humans and rats [15,16]. In the present study, and consistent with previous reports,

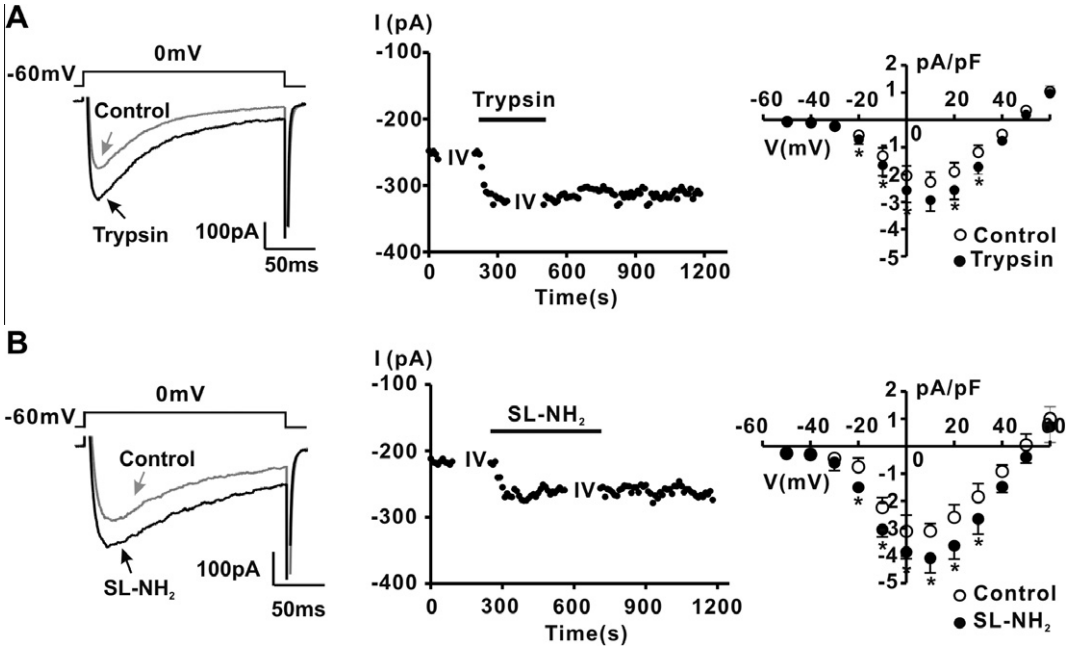

Fig. 3. Effect of PAR2 agonists on $I_{Ca\text{-}L}$ in uterine smooth muscle tissue. (A) Left, representative traces showing effects of 30 nM trypsin on $I_{Ca\text{-}L}$. Middle, time course of 30 nM trypsin (top) on $I_{Ca\text{-}L}$. Right, I–V relationship curve of $I_{Ca\text{-}L}$ in the absence (○) and presence (●) of trypsin. (B) Left, representative traces showing effects of 100 μM SL-NH_2 on $I_{Ca\text{-}L}$. Middle, time course of 100 μM SL-NH_2 on $I_{Ca\text{-}L}$. Right, I–V relationship curve of $I_{Ca\text{-}L}$ in the absence (○) and presence (●) of SL-NH_2.

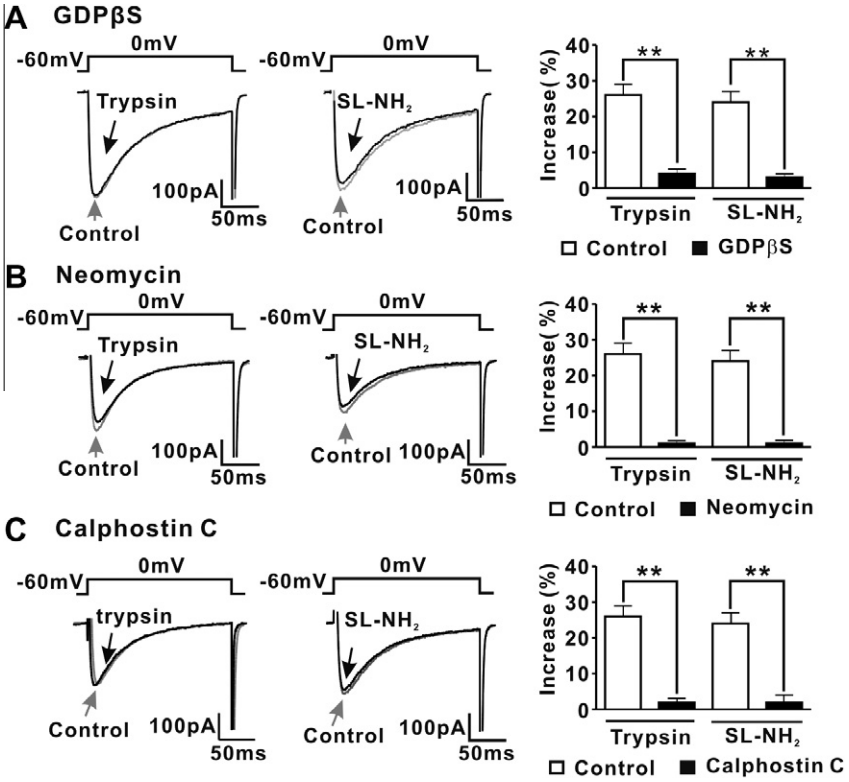

Fig. 4. Stimulatory effect of PAR2 activation on $I_{Ca\text{-}L}$ is GPCR dependent. (A) Representative $I_{Ca\text{-}L}$ trace of 30 nM trypsin or 100 μM SR-NH_2 after 7 min dialysis with 2 mM GDPβS in the pipette solution. Right panel shows a summary of the effects of GDPβS on $I_{Ca\text{-}L}$ increase by 30 nM trypsin and 100 μM SR-NH_2. (B) Representative $I_{Ca\text{-}L}$ trace of 30 nM trypsin or 100 μM SR-NH_2 after 7 min dialysis with 10 μM neomycin in the pipette solution. Right panel shows a summary of the effects of neomycin on $I_{Ca\text{-}L}$ increase by 30 nM trypsin and 100 μM SR-NH_2. (C) Representative $I_{Ca\text{-}L}$ trace of 30 nM trypsin or 100 μM SR-NH_2 after 7 min dialysis with 1 μM calphostin C in the pipette solution. Right panel shows a summary of the effects of calphostin C on $I_{Ca\text{-}L}$ increase by 30 nM trypsin and 100 μM SR-NH_2.

Bay K 8644 greatly augmented SMC in frequency and amplitude (Fig. 2A), and this Bay K 8644 completely occluded the stimulatory effect of PAR-2 (Fig. 2), suggesting that the stimulatory effect of PAR-2 on SMC in pregnant rats may be mediated by modulating L-type voltage-gated Ca^{2+} channels. To make this hypothesis more evident, PAR-2 agonists significantly increased $I_{Ca\text{-}L}$ in an irreversible manner, as measured by a conventional voltage-clamp method (Fig. 3). These data strongly suggest that activation of PAR-2 augments SMC by enhancing Ca^{2+} entry through L-type voltage-gated Ca^{2+} channels. As for irreversible augmentation of $I_{Ca\text{-}L}$ by PAR-2 activation, we cannot suggest a precise explanation about it based on the evidence reviewed in this study. PAR-2 is activated by irreversible proteolysis that results in continuous stimulation; however, cleaved PAR-2 is known to be rapidly ubiquitinated within the C-terminus, which mediates down-regulation of PAR-2 to terminate signaling [26]. A more detailed study is needed to clearly explain this phenomenon.

Although a detailed mechanism of PAR-2 signal transduction has not yet been clearly elucidated, several putative mechanisms have been suggested. PAR-2 are G protein-coupled receptors and couples to $G_{\alpha q/11,}$ which in turn activate phospholipase C, produce inositol 1,4,5-trisphosphate (IP_3) and diacyglycerol (DAG), mobilize Ca^{2+}, and activate protein kinase C (PKC). In addition, PKC enhances $I_{Ca\text{-}L}$ in various smooth muscle, including uterine myometrium [20,21]. Therefore, it is possible that PAR-2-evoked augmentation of SMC may be mediated by activation of PLC and PKC. In the present study, intracellular dialysis of GDPβS (2 mM) nearly completely occluded the augmentation induced by PAR-2 agonists (Fig. 4A), and a blockade of PLC or PKC potently prevented augmentation of SMC by PAR-2 agonists (Fig. 4B and C). These results, therefore, suggest that an increase in $I_{Ca\text{-}L}$ by PAR-2 activation is mediated by PLC and PKC activation via G-proteins.

In conclusion, we have demonstrated that PAR-2 actively regulates SMC during pregnancy by modulating Ca^{2+} influx through L-type voltage-gated Ca^{2+} channels, and that this increase of $I_{Ca\text{-}L}$ is primarily mediated by PLC and PKC activation. These results suggest a cellular mechanism for the pathophysiological effects of PAR-2 activation on SMC during pregnancy and provide basic and theoretical information about developing new agents for the treatment of premature labor and other obstetric complications.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2011.12.154.

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