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Prostaglandin E2 excitatory effects on guinea pig urinary bladder smooth muscle: A novel regulatory mechanism mediated by large-conductance voltage- and Ca^{2+}-activated K^+ channels

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Abstract

Prostaglandin E2 (PGE_2) is an essential signaling molecule involved in the regulation of detrusor smooth muscle (DSM) function. However, the underlying regulatory mechanism by which PGE_2 augments DSM cell excitability and contractility is not well understood. Here, we investigated whether PGE inhibits the large conductance voltage- and Ca^{2+}-activated K^+ (BK) channels in guinea pig DSM, thereby increasing DSM excitability and contractility. We used a multidisciplinary experimental approach including amphotericin–B perforated patch-clamp electrophysiology and live-cell Ca^{2+} imaging in native freshly-isolated DSM cells, isometric tension recordings of intact DSM strips, and pharmacological tools to investigate BK channel regulation by PGE_2 in guinea pig DSM. PGE_2 increased the spontaneous phasic contractions of isolated DSM strips in a concentration-dependent manner (10 nM-10 μM). BK channel inhibition with paxilline (1 μM) attenuated the PGE_2-induced DSM phasic contractions, suggesting that BK channels are involved in the mechanism of PGE_2-induced DSM contractions. PGE_2 (10 μM) increased the intracellular Ca^{2+} levels in freshly-isolated DSM cells. PGE_2 (10 μM) also caused an inhibition of the amplitude and frequency of spontaneous transient BK currents in DSM cells. Moreover, PGE_2 (10 μM) did not affect the amplitude of whole cell steady-state BK currents in DSM cells. Our findings provide strong experimental evidence that PGE_2 leads to an inhibition of the spontaneous transient BK currents, elevation of intracellular Ca^{2+} levels in freshly-isolated DSM cells, and augmentation of DSM phasic contractions. Thus, we have revealed a novel mechanism that BK channels mediate PGE_2-induced contractions in guinea pig DSM.

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CONFLICTS OF INTEREST
The authors declare no conflicts of interest.

Keywords

Prostaglandin E2; detrusor; patch-clamp; Ca^{2+} imaging

1. INTRODUCTION

Prostaglandin E_2 (PGE_2) is an essential signaling molecule differentially regulating smooth muscle function depending on the expression of E-prostanoid (EP) receptor subtypes-EP receptor subtype 1 (EP_1), EP receptor subtype 2 (EP_2), EP receptor subtype 3 (EP_3), and EP receptor subtype 4 (EP_4) (Narumiya et al., 1999). PGE_2 is coupled to divergent signaling pathways for distinct cellular and physiological functions in smooth muscles, including detrusor smooth muscle (DSM) (Narumiya et al., 1999). PGE_2 has been shown to be involved in the regulation of DSM contractility in both rodents and humans (Anderson, 1993; Creed and Callahan, 1989; Klausner et al., 2011; Kobayter et al., 2012; McCafferty et al., 2008). It is synthesized in the urothelial and muscle layers of the urinary bladder (Brown et al., 1980; Klausner et al., 2011; Masunaga et al., 2006; Park et al., 1999). Immunohistochemistry analyses revealed that EP_1 and EP_2 receptors are expressed in the urothelium, lamina propria, and DSM (de Jongh et al., 2007; Rahnama'i et al., 2010).

Differential effects of PGE_2 on smooth muscle function have been reported (Anderson, 1993; Coleman and Sheldrick, 1989; Ishizuka et al., 1995; Kobayter et al., 2012; Zhu et al., 2002). PGE_2 has either stimulatory or inhibitory effects depending on the species examined or the tissue or cell types being investigated. PGE_2 increases the spontaneous activity of mouse DSM strips, whereas in human urethral smooth muscle it causes relaxation (Anderson, 1993; Kobayter et al., 2012). In mouse DSM, PGE_2 has been reported to cause cell membrane depolarization, to increase Ca^{2+} oscillations, and to potentiate phasic contractions (Kobayter et al., 2012). In human airway smooth muscle, PGE_2 has been demonstrated to induce either stimulatory or inhibitory effects on contractions depending on the concentration applied (Coleman and Sheldrick, 1989). In addition, PGE_2 has been suggested to cause relaxation of coronary artery smooth muscle by activating the large conductance voltage- and Ca^{2+}-activated K^+ (BK) channels (Zhu et al., 2002).

Among all known ion channels, BK channels are the most critical regulators of DSM function in both rodents and humans (Hristov et al., 2011; Petkov, 2012; Petkov and Nelson, 2005). BK channels are activated by rapid and localized Ca^{2+} releases from sarcoplasmic reticulum ryanodine receptors, known as "Ca^{2+} sparks", which elicit transient BK currents (TBKCs), also known as spontaneous transient outward currents (STOCs), in DSM cells (Herrera et al., 2001; Herrera and Nelson, 2002; Hristov et al., 2011; Petkov, 2012; Petkov and Nelson, 2005). In human DSM, direct inhibition of the BK channels with its selective blockers, iberiotoxin or paxilline, leads to membrane depolarization, activation of L-type voltage-gated Ca^{2+} (Ca_V) channels, and potentiation of DSM contractions (Hristov et al., 2011). By contrast, activation of BK channels with pharmacological openers such as NS1619, hyperpolarizes the membrane potential, increases BK channel open probability and limits the Ca^{2+} entry via Ca_V channels, thereby causing DSM relaxation (Hristov et al., 2011; Hristov et al., 2012; Malysz et al., 2013; Petkov, 2012). A recent report from our

group revealed that activation of M_3 muscarinic receptors leads to inhibition of BK channel activity via a Ca^{2+}-dependent mechanism, thereby increasing DSM excitability and contractility in rat DSM (Parajuli and Petkov, 2013). Since PGE_2 has been shown to depolarize the cell membrane potential and increase mouse DSM contractility (Kobayter et al., 2012), we hypothesize that BK channels play a critical role in mediating the PGE_2 stimulatory effects on DSM excitability and contractility.

In this study, we sought to elucidate whether BK channels mediate the PGE_2 stimulatory effects on guinea pig DSM excitability and contractility. For this purpose, we employed a multidisciplinary experimental approach including isometric DSM tension recordings, live-cell Ca^{2+} imaging, and perforated whole cell patch-clamp electrophysiology at both cellular (freshly-isolated single cells) and tissue levels (intact DSM strips). For the first time, our data revealed that PGE_2 leads to inhibition of TBKCs, elevation of intracellular Ca^{2+} levels, and potentiation of guinea pig DSM contractility.

2. MATERIALS AND METHODS

2.1. DSM tissue collection and preparation

22 male Hartley Albino guinea pigs (Charles River Laboratories, Raleigh, NC) of average weight 473.7±16.3 g were euthanized by CO_2 inhalation. The urinary bladders were dissected in accordance to the animal use protocol #1747, and reviewed and approved by the Institutional Animal Care and Use Committee at the University of South Carolina. Urinary bladders were cut open longitudinally from the lumen and stored in ice-cold dissection solution. DSM strips ~5-6 mm long and ~2-3 mm wide were dissected from the bladder wall and the urothelium and lamina propria were removed.

2.2. DSM single cell isolation

Guinea pig DSM single cells were enzymatically isolated using papain and collagenase as previously described (Hristov et al., 2013; Parajuli and Petkov, 2013). Single DSM cells were used for patch-clamp and live-cell Ca^{2+} imaging studies within 12 h after isolation.

2.3. Isometric DSM tension recordings

Isometric DSM tension recordings of freshly-isolated guinea pig DSM strips (~5-6 mm long and ~2-3 mm wide) were conducted as described previously (Hristov et al., 2013; Smith et al., 2013). Cumulative PGE_2 concentrations (10 nM-10 μM) were applied at 15-min intervals into the baths in the presence or absence of paxilline (1 μM), a selective inhibitor of BK channels.

2.4. Live-cell Ca^{2+} imaging

Intracellular Ca^{2+} imaging experiments were performed using the ratiometric dye fura 2-AM in freshly-isolated DSM single cells as described previously (Hristov et al., 2013; Smith et al., 2013).

2.5. Patch-clamp recordings

The amphotericin-B perforated whole cell patch-clamp technique was used to record TBKCs and voltage-step depolarization-induced whole cell steady-state BK currents in freshly-isolated DSM single cells as described previously (Hristov et al., 2013; Parajuli and Petkov, 2013). TBKCs were recorded at a holding potential of −40 mV. Voltage-step-induced whole cell BK currents were recorded by holding the DSM cells at −70 mV and voltage-depolarization applied from −40 mV to +80 mV in increments of 20 mV with 200 ms duration, and then cells were repolarized back to −70 mV. All patch-clamp recordings were performed at room temperature (22-23° C).

2.6. Solutions and drugs

The Ca^{2+}-free dissection solution, the extracellular bath solution used in the perforated patch-clamp and Ca^{2+} imaging experiments, and the patch-clamp pipette solution for perforated whole cell patch-clamp experiments were prepared as described previously (Hristov et al., 2013; Smith et al., 2013). Freshly-dissolved amphotericin-B (200 g/ml) in dimethyl sulfoxide (DMSO) was added to the pipette solution before the experiments and replaced every 1-2 h. PGE_2 and fura 2-AM were purchased from Sigma-Aldrich (St. Louis, MO) and were dissolved in ethanol and DMSO as stock solutions, respectively. Paxilline was purchased from Tocris Bioscience (Bristol, UK) and was dissolved in DMSO. The DMSO concentration in the bath solution did not exceed 0.1%.

2.7. Data analysis and statistics

The effects of PGE_2 on DSM contraction parameters or the amplitude and frequency of TBKCs were analyzed using MiniAnalysis software (Synaptosoft, Inc., Fort Lee, NJ) and then were normalized to control values and were expressed in percentages (%). The effect of PGE_2 on voltage-step depolarization-evoked steady-state whole cell BK current was analyzed by Clampfit 10.2 software (Molecular Device, Union City, CA). The mean value of the last 50 ms of the 200 ms pulse from the average of 6-10 files recorded over 8-10 min (performed every 1 min) were calculated before and after the application of 10 μM PGE_2. The last 5 min of at least 8-10 min stable voltage-clamp recordings prior to the application of PGE_2 were used as a control and the last 5 min of continuous recordings of at least 10-15 min after the application of PGE_2 were used to evaluate their effects on TBKCs from DSM cells. Statistical analysis was performed with GraphPad Prism 4.03 software (GraphPad Software, Inc., La Jolla, CA). The data are presented as mean±S.E.M. for the **n** (the number of cells or strips) isolated from **N** (the number of guinea pigs). Data were illustrated by using Corel Draw Graphic Suite X3 software (Corel Co., Mountain View, CA) and GraphPad Prism 4.03 software. Statistical analysis was performed using a two-tailed paired Student's t-test or two-way ANOVA followed by Bonferroni post-hoc test. A P value <0.05 was considered to be statistically significant.

3. RESULTS

3.1. PGE_2 increases the spontaneous phasic contractions of freshly-isolated DSM strips

We sought to explore whether PGE_2 regulates DSM spontaneous phasic contractions. As illustrated in **Fig. 1**, isolated DSM strips exhibiting spontaneous phasic contractions were exposed to cumulative concentrations of PGE_2 (10 nM-10 μM). PGE_2 (10 nM-10 μM) significantly increased the spontaneous phasic contraction amplitude and muscle force integral in a concentration-dependent manner (n=8, N=5; P<0.05; **Fig. 1A, C, D**). At the highest concentration used of 10 μM, PGE_2 increased DSM spontaneous phasic contraction amplitude by 488.5±114.4% and the muscle force integral by 698.4±163.6% (n=8, N=5; P<0.05, **Fig. 1**).

To further evaluate whether PGE_2 stimulatory effects on DSM spontaneous phasic contractions were mediated by the BK channels, we investigated the effects of PGE_2 (10 nM-10 μM) on DSM contractility in the presence of the BK channel selective inhibitor, paxilline (1 μM). As shown in **Fig. 1B**, we found that paxilline (1 μM) attenuated the PGE_2 stimulatory effects on DSM phasic contraction amplitude and muscle force integral. Pretreatment of DSM strips with paxilline reduced the PGE_2 (10 μM)-induced contraction amplitude and contraction muscle force integral from 488.5±114.4% to 194.2±24.7% and from 698.4±163.6% to 225.2±55.2%, respectively (n=8, N=5; P<0.05, **Fig. 1C, D**). We observed attenuation in the concentration-response curves for PGE_2 effects on the spontaneous phasic contraction amplitude and the muscle force integral in the presence of paxilline (**Fig. 1C, D)**. These data indicate that BK channels are involved in mediating the PGE_2 stimulatory effects on DSM spontaneous phasic contractions.

3.2. PGE increases the intracellular Ca^{2+} levels in freshly-isolated DSM cells

Since our data showed that PGE_2 dramatically increased DSM spontaneous phasic contractions, we expect that PGE affects the intracellular Ca^{2+} levels in freshly-isolated DSM cells. Using the ratiometric dye fura 2-AM and live-cell Ca^{2+} imaging, we measured the intracellular Ca^{2+} levels in DSM cells as shown in **Fig. 2A**-**B**. In DSM cells, PGE_2 (10 μM) significantly increased the fura 2 fluorescence ratio 340/340 of intracellular Ca^{2+} levels from its control value of 0.76±0.1 to 1.0±0.2 (P<0.05; n=6, N=6; **Fig. 2**).

3.3. PGE_2 inhibits the amplitude and frequency of TBKCs in freshly-isolated DSM cells

In the next experimental series, we studied the effect of PGE_2 on the amplitude and frequency of TBKCs in DSM cells using the amphotericin-B perforated whole cell patch-clamp technique. The average cell capacitance of all 18 DSM cells isolated from 9 guinea pigs used in the present study was 35.16±4.4 pF, consistent with previous reports (Herrera et al., 2001; Herrera and Nelson, 2002). As shown in **Fig. 3A**-**C**, the treatment of DSM cells with PGE_2 (10 μM) significantly inhibited the amplitude and frequency of TBKCs by 27.3±9.5% and 30.1±12.8%, respectively (n=12, N=7; P<0.05). These results indicate that PGE_2 increases DSM contractility by inhibiting TBKCs in DSM cells. Next, we sought to elucidate whether PGE_2 affects the steady-state whole cell BK currents in freshly-isolated DSM cells.

3.4. PGE_2 does not affect the steady-state whole cell BK currents in freshly-isolated DSM cells

Previous studies from our group have shown that in animal and human DSM cells, the voltage-step-induced steady-state whole cell currents are primarily mediated by the BK channel activity (Hristov et al., 2011; Petkov and Nelson, 2005). Since PGE_2 has shown statistically significant inhibitory effects on the amplitude and frequency of TBKCs, we sought to explore whether PGE_2 also affects the voltage-step-induced steady-state BK currents in guinea pig DSM cells. We measured the effects of PGE_2 (10 μM) on voltage-step depolarization-induced BK currents. PGE_2 (10 μM) did not alter significantly the voltage-step depolarization-induced BK currents (n=6, N=6; P>0.05, **Fig. 4**). As shown in **Fig. 4B**, at the highest depolarization voltage applied (+80 mV), the steady-state whole cell BK currents were 12.5±0.9 pA/pF and 13.4±1.0 pA/pF in the absence and presence of PGE_2 (10 μM), respectively (n=6, N=6; P>0.05; **Fig. 4B**). These results suggest that unlike the TBKCs, PGE_2 does not affect the steady-state BK channel currents in freshly-isolated guinea pig DSM cells.

4. DISCUSSION

In the present study, for the first time, we have revealed a mechanism that BK channels mediate PGE_2-induced excitability and contractility in guinea pig DSM. We provide evidence that PGE_2 causes: 1) increase in DSM spontaneous phasic contractions in a BK channel-dependent manner, 2) elevation of the intracellular Ca^{2+} concentration in freshly-isolated DSM cells, and 3) inhibition of the amplitude and frequency of TBKCs in freshly-isolated DSM cells.

Our experimental data from isometric DSM tension recordings showed that PGE_2 increased the spontaneous phasic contractions of isolated DSM strips (**Fig. 1A**). PGE_2-induced stimulatory effects on spontaneous phasic contractions were significantly attenuated by the BK channel blocker paxilline, supporting that the observed PGE_2 stimulatory effects on DSM contractility were mediated by the BK channels (**Fig. 1B**-**E**). A recent study in mouse DSM reported that PGE_2 increased DSM contractions by activation of L-type Ca_V channels (Kobayter et al., 2012). The inhibition of BK channels indirectly activates the L-type Ca_V channels and increases the global Ca^{2+} concentration to promote DSM contractility (Petkov, 2012). Indeed, in the presence of paxilline, PGE_2 had less pronounced stimulatory effects on DSM contractions as compared with PGE_2-induced phasic contractions (**Fig. 1**).

In other non-DSM smooth muscle tissues including that of the human airway and urethra, PGE_2-induced responses were variable (Anderson, 1993; Coleman and Sheldrick, 1989). The diverse cellular and tissue-specific responses to PGE_2 are suggested to be a result of the activation of multiple EP receptor subtypes as well as their variable distribution (Narumiya et al., 1999). Our experimental data demonstrate that activation of EP receptors with PGE_2 leads to an inhibition of TBKC activity in guinea pig DSM, thereby increasing DSM excitability and contractility (**Fig. 1**). We observed PGE stimulatory effects on intracellular Ca^{2+} levels in DSM single cells and spontaneous phasic contractions of isolated DSM strips (**Figs. 1 and 2**). One of the prototypic signaling pathways involving PGE -induced elevation of intracellular Ca^{2+} levels and associated DSM contractility is thought to be a result of EP_1

receptor activation (Bos et al., 2004). EP_1 receptors couples to G_q proteins and stimulates phospholipase C, which hydrolyzes phosphatidylinositol-4,5-bisphosphate (PIP_2) to generate diacylglycerol and inositol-1,4,5-trisphosphate (Bos et al., 2004). The binding of inositol-1,4,5-trisphosphate to inositol-1,4,5-trisphosphate receptors leads to a release of Ca^{2+} from the sarcoplasmic reticulum, which elevates intracellular Ca^{2+} levels. Furthermore, diacylglycerol, another PIP_2 product, may activate protein kinase-C (PKC). Recently, our group has reported that activation of PKC with phorbol 12-myristate 13-acetate (PMA) leads to an elevation of intracellular Ca^{2+} levels, potentiation of DSM phasic contractions, and inhibition of the amplitude and frequency of TBKCs of DSM cells (Hristov et al., 2013). Consistent with this mechanism, PGE_2 most likely stimulates EP_1 receptors and then activates PKC. PKC may cause a direct inhibition of ryanodine receptors or a reduction in sarcoplasmic reticulum Ca^{2+} load resulting in an inhibition of the amplitude and frequency of DSM TBKCs (Hristov et al., 2013; Petkov, 2012). In the present study, since activation of EP receptors with PGE leads to an elevation of intracellular Ca^{2+} levels, potentiation of DSM phasic contractions, and an inhibition of the amplitude and frequency of TBKCs consistent with PKC-induced effects on guinea pig DSM excitability and contractility, we suggest that PGE_2 stimulatory effects on guinea pig DSM excitability and contractility may involve an EP_1 receptor-PKC-BK channel-dependent mechanism.

Since our data on DSM contractility revealed that PGE_2 stimulatory effects on DSM spontaneous phasic contractions are associated with reduction of TBKC channel activity, we further sought to elucidate whether the observed PGE_2 effects on DSM contractions were associated with elevation of intracellular Ca^{2+} levels in DSM cells. In human DSM cells, BK channel activation with NS1619 leads to a reduction of intracellular Ca^{2+} levels indicating a relationship between BK channel activation and cytosolic Ca^{2+} level (Hristov et al., 2012). In DSM isolated strips, PGE_2 caused membrane potential depolarization and enhanced nerve- and acetylcholine-induced contractions (Creed and Callahan, 1989). It was suggested that these effects were mediated by intracellular Ca^{2+} mobilization (Creed and Callahan, 1989). Furthermore, inhibition of DSM BK channel activity leads to membrane depolarization, increased intracellular Ca^{2+} levels, and promotion of DSM contractions (Petkov, 2012). Those previous reports are in support of the present findings as PGE_2 significantly elevated the intracellular Ca^{2+} levels in DSM single cells and increased the spontaneous phasic contractions of isolated DSM strips. Our findings from Ca^{2+} imaging experiments further indicate that PGE_2 stimulatory effects on DSM contractility are associated with elevation of intracellular Ca^{2+} levels in DSM cells (**Figs. 1 and 2**).

In the present study, we investigated the mechanism by which PGE_2 causes stimulation of DSM cell excitability with the help of the perforated patch-clamp technique. The perforated mode of the patch-clamp technique has the advantage of preserving the intrinsic intracellular signaling pathways, thus maintaining the native physiological environment for studying DSM BK channels. To the best of our knowledge, this is the first time that patch-clamp electrophysiology and live-cell Ca^{2+} imaging has been performed on freshly-isolated native guinea pig DSM cells in order to evaluate the effect of PGE on intracellular Ca^{2+} levels and BK channel activity. Under these particular physiological conditions, PGE_2 had a statistically significant inhibitory effect on the amplitude and frequency of TBKCs without

affecting the amplitude of steady-state whole cell BK currents (**Figs. 3 and 4**). In DSM, Ca^{2+} release from sarcoplasmic reticulum ryanodine receptors activates TBKCs without influencing steady-state BK currents indicating the sarcoplasmic reticulum Ca^{2+} release via ryanodine receptors is critical in regulating BK channels (Herrera et al., 2000, 2001; Hristov et al., 2011; Petkov and Nelson, 2005). It has been shown that inhibition of ryanodine receptors with ryanodine eliminates the TBKCs without affecting the amplitude of steady-state BK currents (Hristov et al., 2011; Petkov and Nelson, 2005). In the present study, the observed PGE_2 inhibitory effects on the amplitude and frequency of DSM TBKCs may be attributed to a direct inhibition of the ryanodine receptors or a reduction in sarcoplasmic reticulum Ca^{2+} load (Petkov, 2012).

Reports demonstrate that PGE_2 concentrations are dramatically increased during bladder pathophysiological conditions (Aoki et al., 2009). Intravesical application of PGE_2 has been shown to induce detrusor overactivity indicating a potential role of PGE_2 in bladder pathophysiology (Schroder et al., 2004). Further, urinary levels of PGE_2 were significantly increased in patients with overactive bladder symptoms indicating that PGE_2 may be linked to bladder pathophysiology, thus urine PGE_2 levels could be used as biomarkers of bladder dysfunction (Aoki et al., 2009; Kim et al., 2006; Rahnama'i et al., 2012). Urinary retention due to detrusor underactivity is another form of bladder dysfunction for which effective pharmacotherapies are lacking. A study on patients with detrusor underactivity, who were already receiving cholinergic stimulation treatment showed that PGE_2 had an additive or synergistic effect on cholinergic stimulation-induced responses (Desmond et al., 1980). Another study on patients with detrusor underactivity suggested that PGE_2 when combined with bethanechol may be considered for treatment of patients with detrusor underactivity (Hindley et al., 2004). As pharmacological activation of EP receptors with PGE_2 increases DSM cell excitability and contractility, the present study suggests that EP receptor agonists, or agents that stimulate PGE_2 production, may prove to be beneficial for the treatment of detrusor underactivity.

CONCLUSIONS

Our findings provide evidence that PGE_2 leads to inhibition of TBKC activity, elevation of intracellular Ca^{2+} levels in DSM cells, and increase in DSM spontaneous phasic contractions. Therefore, we have revealed a novel mechanism by which BK channels mediate PGE_2-induced stimulatory effects on guinea pig DSM excitability and contractility.

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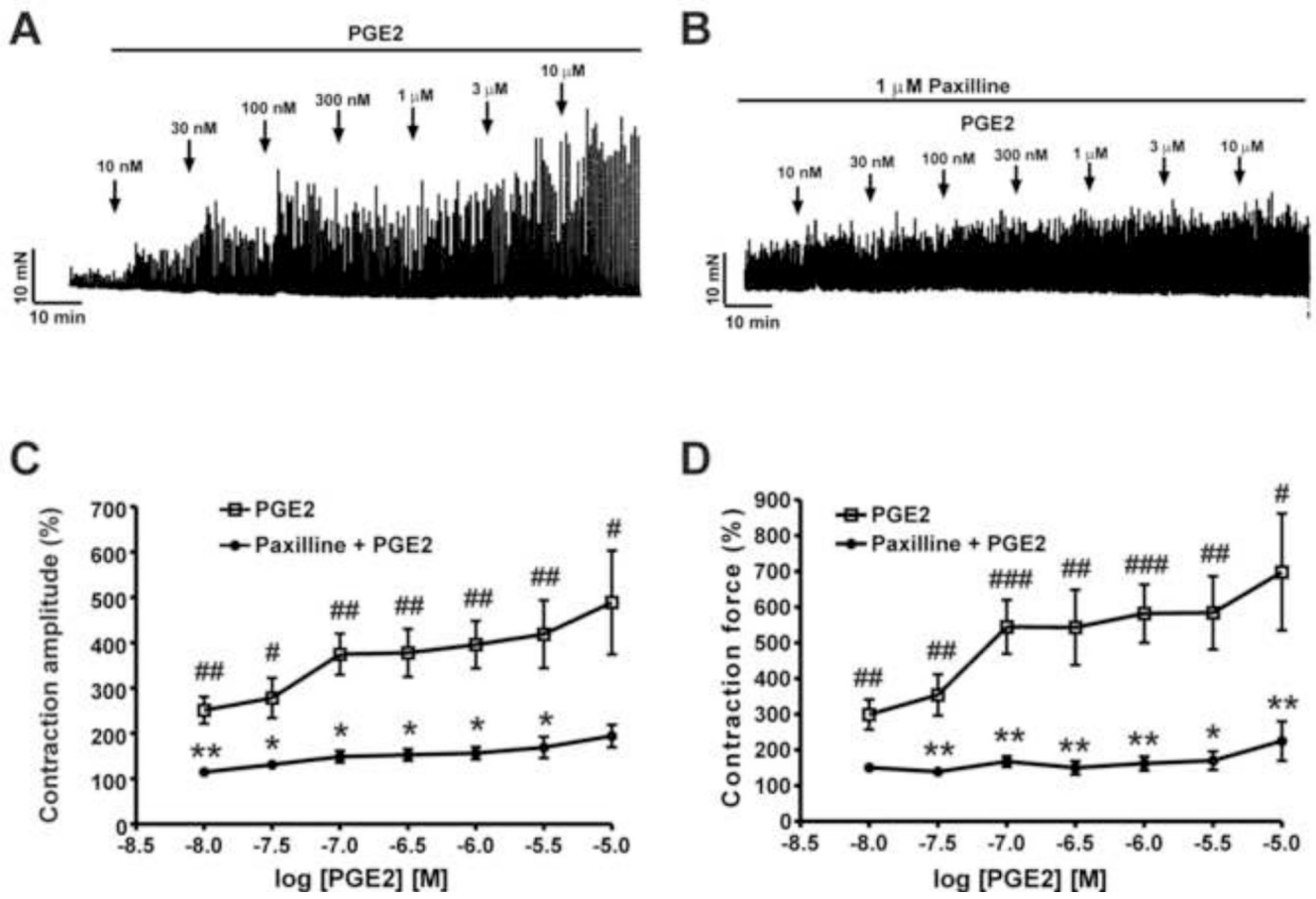

Fig. 1. PGE_2 significantly increases the spontaneous phasic contractions of guinea pig isolated DSM strips. A and B)

Representative original recordings showing the contractile effects of PGE_2 (10 nM-10 μM) on spontaneous phasic contractions of isolated DSM strips in the absence (**A**) or presence (**B**) of paxilline (1 μM). **C and D)** Cumulative concentration-response curves for PGE_2 on spontaneous phasic contraction amplitude and muscle force integral of DSM strips in the presence or absence of 1 μM paxilline (n=8, N=5; $^{\#}P<0.05$, $^{\#\#}P<0.01$, and $^{\#\#\#}P<0.001$ PGE_2 vs. control, *P<0.05 and **P<0.01 vs. paxilline).

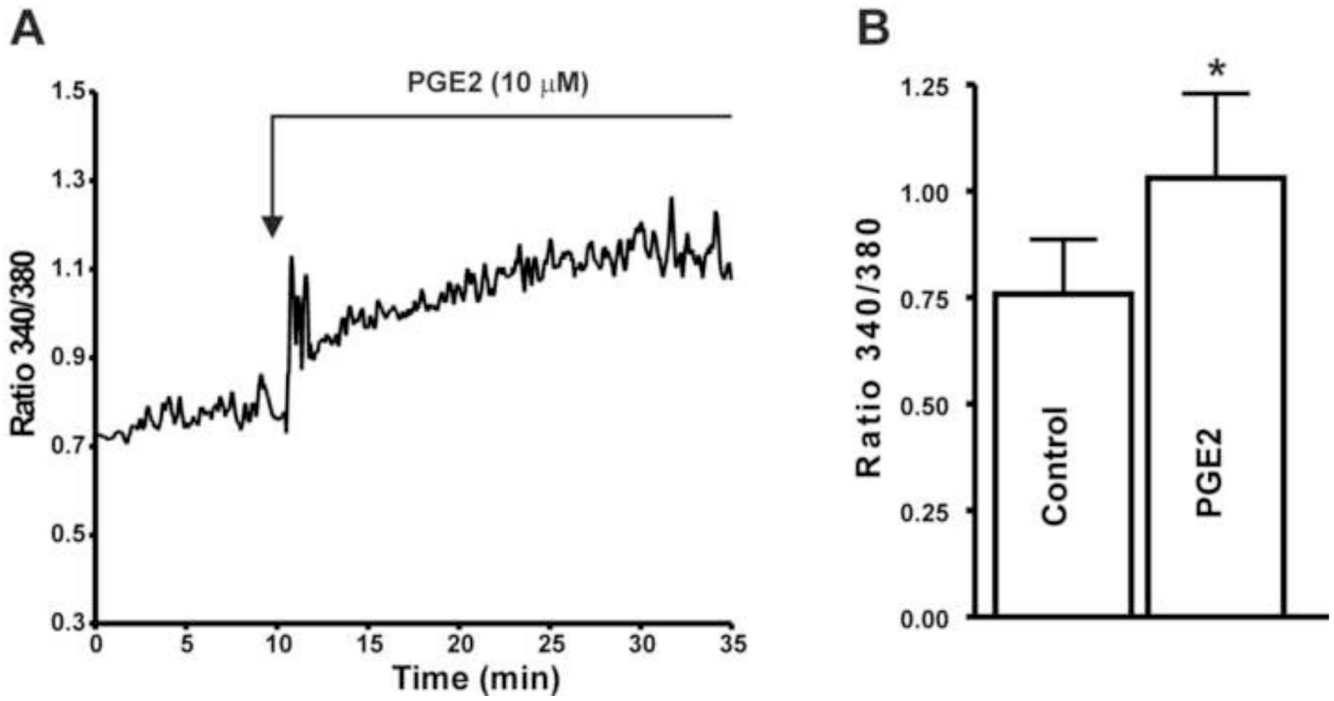

Fig. 2. PGE_2 increases the intracellular Ca^{2+} levels in native freshly-isolated guinea pig DSM cells. A)

A representative trace of fura 2 fluorescence ratio illustrating that PGE_2 (10 μM) increases the intracellular Ca^{2+} levels in a DSM cell. **B)** Summary data depicting the DSM cell intracellular Ca^{2+} levels in the presence or absence of PGE_2 (10 μM) (n=6, N=6; * $P<0.05$).

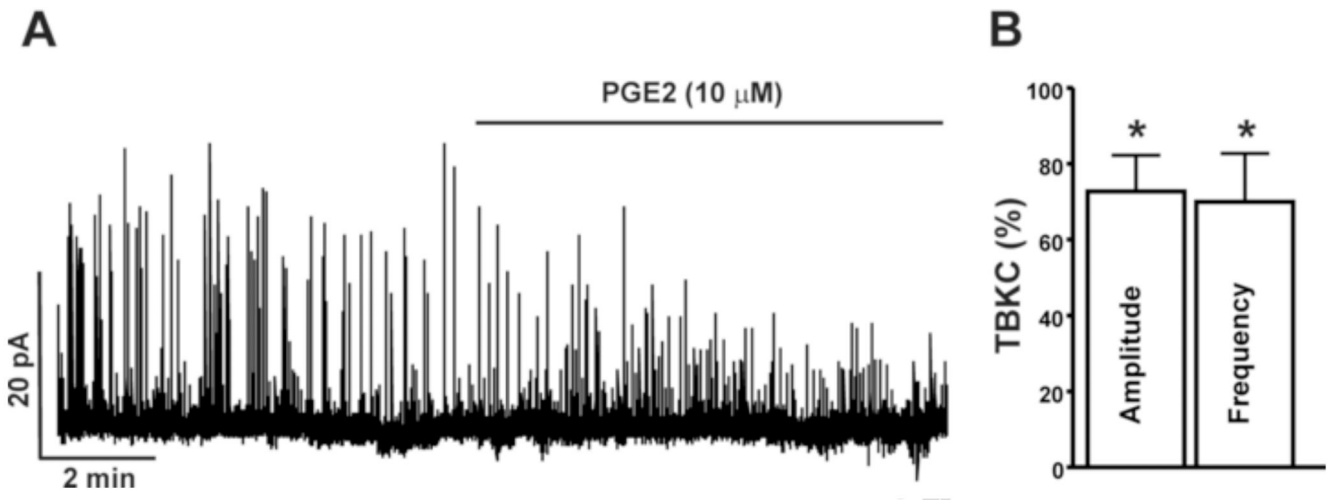

Fig. 3. PGE_2 inhibits TBKCs in freshly-isolated guinea pig DSM cells. A)
A representative recording illustrating the PGE_2 (10 μM) inhibitory effects on the TBKC amplitude and frequency in a freshly-isolated DSM cell. **B)** Summary data illustrating the inhibitory effect of PGE_2 (10 μM) on the amplitude and frequency of TBKCs (n=12, N=7; *P<0.05). The average amplitude and frequency of TBKCs before PGE_2 application (control) were taken to be 100% and data were normalized to controls.

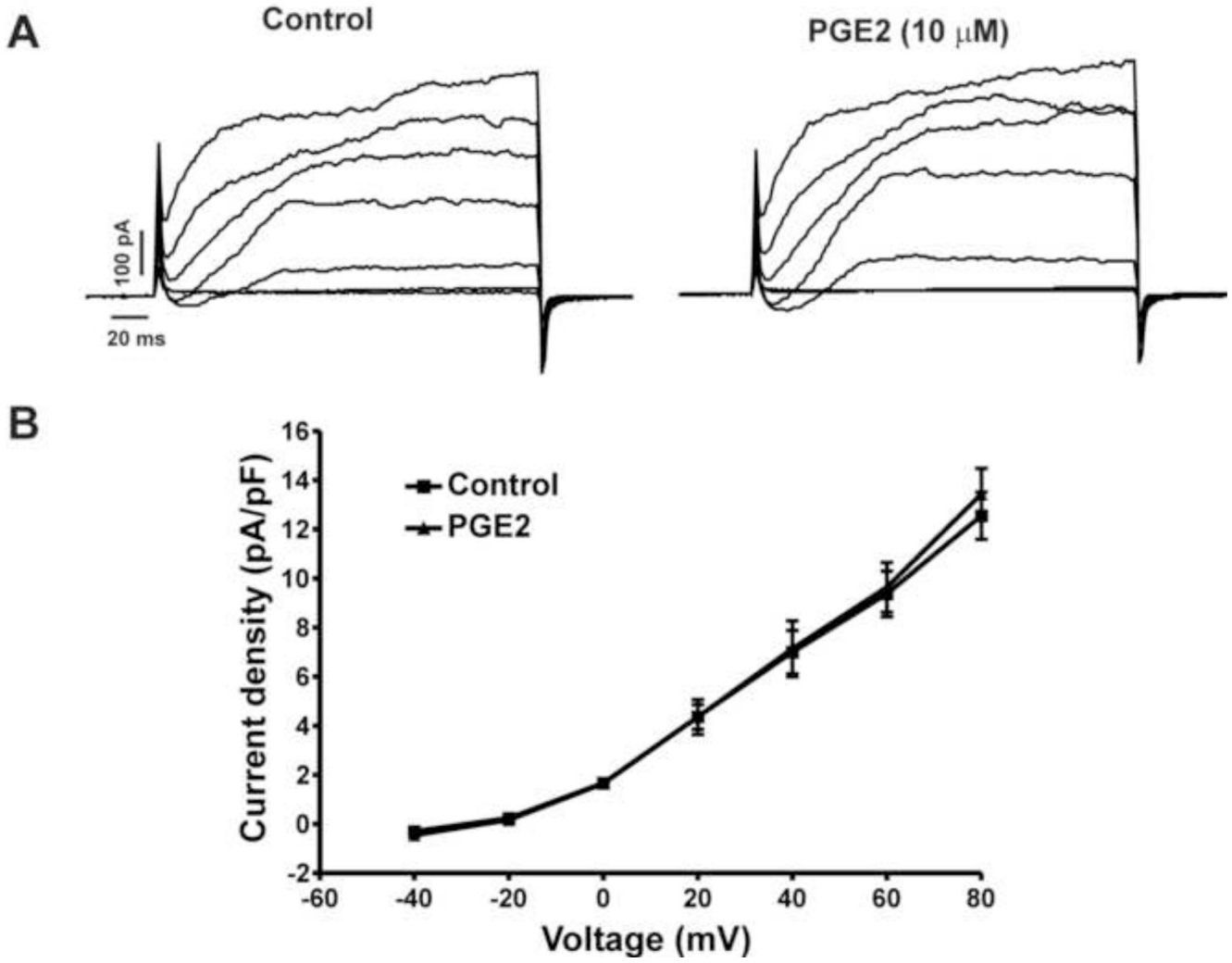

Fig. 4. PGE_2 does not affect the steady-state whole cell BK currents in freshly-isolated guinea pig DSM cells. A)

Representative voltage-clamp recordings illustrating that PGE_2 (10 μM) does not affect the steady-state BK currents at depolarization voltages from −40 mV to +80 mV. **B)** Current-voltage relationship curves depict the lack of PGE_2 effect on the steady-state whole cell BK currents in DSM cells (n=6, N=6; P>0.05).