Biochemical and Biophysical Research Communications 439 (2013) 363–368

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

IP3 decreases coronary artery tone via activating the BK_{Ca} channel of coronary artery smooth muscle cells in pigs

Yan Yang, Peng-Yun Li, Jun Cheng, Fang Cai, Ming Lei, Xiao-Qiu Tan, Miao-Ling Li, Zhi-Fei Liu, Xiao-Rong Zeng *

Key Laboratory of Medical Electrophysiology, Ministry of Education, The Institute of Cardiovascular Research, Luzhou Medical College, Luzhou 646000, China

ARTICLE INFO

Article history:
Received 13 August 2013
Available online 4 September 2013

Keywords:
Large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channel
Inositol trisphosphate (IP3)
Vascular smooth muscle
Coronary artery

ABSTRACT

Large conductance Ca^{2+}-activated K^+ channel (BK_{Ca}) is a potential target for coronary artery-relaxing medication, but its functional regulation is largely unknown. Here, we report that inositol trisphosphate (IP3) activated BK_{Ca} channels in isolated porcine coronary artery smooth muscle cells and by which decreased the coronary artery tone. Both endogenous and exogenous IP3 increased the spontaneous transient outward K^+ currents (STOC, a component pattern of BK_{Ca} currents) in perforated and regular whole-cell recordings, which was dependent on the activity of IP3 receptors. IP3 also increased the macroscopic currents (MC, another component pattern of BK_{Ca} currents) via an IP3 receptor- and sarcoplasmic Ca^{2+} mobilization-independent pathway. In inside-out patch recordings, direct application of IP3 to the cytosolic side increased the open probability of single BK_{Ca} channel in an IP3 receptor-independent manner. We conclude that IP3 is an activator of BK_{Ca} channels in porcine coronary smooth muscle cells and exerts a coronary artery-relaxing effect. The activation of BK_{Ca} channels by IP3 involves the enhancement of STOCs via IP3 receptors and stimulation of MC by increasing the Ca^{2+} sensitivity of the channels.

1. Introduction

Decreasing coronary artery tone is one of the leading strategies in treating ischemic heart diseases. The large conductance Ca^{2+}-activated K^+ (BK_{Ca}) channel may serve as a switch that decides whether vasoactive factors induce vasoconstriction (via Ca^{2+} signaling) or vasorelaxation (via membrane hyperpolarization). The BK_{Ca} currents consist of the spike-like spontaneous transient outward K^+ currents (STOC) and macroscopic currents (MC) under the whole-cell voltage clamp configuration [1,2]. Given their abundant expression in blood vessels and characteristic large conductance, BK_{Ca} channels are supposed to participate in membrane potential stabilization in vascular smooth muscle cells (VSMCs). In response to fluctuations in membrane potential and intracellular Ca^{2+} concentration, BK_{Ca} channels act as a negative regulator of vascular tone[3], relaxing blood vessels such as coronary arteries [4,5]. However, endogenous factors that regulate BK_{Ca} channel activities are not well identified, especially in the coronary arteries.

Phospholipids, the important constituents of cell membranes, have been known to alter the function of BK_{Ca} channels [3]. Among the membrane phospholipids, phosphatidylinositol 4, 5-bisphosphate (PIP_2) has been extensively investigated in particular because it serves as a precursor for IP3 and diacylglycerol (DAG). IP3 is the natural ligand of IP3 receptor (IP3R) and mobilizes sarcoplasmic Ca^{2+} when binding to IP3R. The mobilization of Ca^{2+} and activation of related other activated factors eventually influence many ion channels at the plasma membrane [6,7]. BK_{Ca} channel and IP3R are expressed in VSMCs and IP3 has been reported to activate BK_{Ca} channels via type 1 IP3 receptor (IP3R1) in rat and mouse cerebral arterial SMCs [8]. However, the role of IP3 in the electrophysiological activity of coronary artery SMCs is rarely studied. Results obtained from other arteries such as cerebral arteries cannot be directly used to extrapolate the physiological mechanisms in coronary arteries [9]. In order to figure out how IP3 activate the BK_{Ca} channels of coronary artery SMCs and thereafter relax the coronary arteries, the present study was designed to do the measurement at cellular and tissue levels using isolated porcine coronary SMCs and coronary artery rings, respectively. The results demonstrate that IP3 activates BKCa channels and exerts a coronary artery-relaxing effect. IP3 enhances the STOC and MC components of BK_{Ca} currents via different mechanisms.

* Corresponding author. Address: Institute of Cardiovascular Research, Luzhou Medical College, Luzhou 646000, Sichuan Province, China. Fax: +86 830 3161258.
E-mail address: lyxjd7151@163.com (X.-R. Zeng).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.08.079

2. Materials and methods

2.1. Preparation of porcine coronary artery smooth muscle cells

Coronary arterial tissues were collected from a total of 53 fresh adult porcine hearts obtained from a slaughterhouse. Single VSMCs were enzymatically isolated with procedure as described previously [2] and in the online Supplementary methods.

2.2. Whole-cell and single channel recordings

The spontaneous transient outward K^+ current (STOC) and the macroscopic current (MC), the two major patterns of BK_{Ca} currents, were detected in a regular whole-cell voltage clamp configuration and also in some cases in a perforated whole-cell configuration. Single-channel current recordings were conducted under the inside-out configuration with the techniques as described previously [2,10] and in the online Supplementary methods.

2.3. Vasomotor assay

The left anterior descending coronary arteries (LAD) were used for the vasomotor assay using the procedure as described in the online Supplementary methods.

2.4. Statistical analysis

Data are expressed as mean ± SEM. n is the number of cells tested. Paired and grouped t-tests were used for the statistical analysis. $P < 0.05$ was considered statistically significant.

3. Results

3.1. IP3 enhances the STOC component of BK_{Ca} currents via activating IP3R in porcine coronary smooth muscle cells

The STOC was detected over a wide range of membrane potentials under whole-cell conditions in SMCs of porcine coronary artery. At a holding potential of –20 mV, the amplitude (10–130 pA) and frequency (0.02–2 Hz) of STOC varied widely from cell to cell and with time. The STOC was suppressed by IbTX (200 nM), a selective BK_{Ca} channel blocker (Supplementary Fig. S1A).

To further investigate the potential modulatory effect of IP3 on the STOC, we evaluated the contribution of IP3R in STOC generation using perforated whole-cell recordings, in which the endogenous IP3 is kept at a physiological intracellular level without leaking out from intracellular space and therefore can activate the IP3R located at the intracellular side. Extracellular application of 10 μM xestospongin-C (XeC, an IP3R blocker which is membrane permeable) decreased the STOC. The frequency of STOC was reduced from 1.41 ± 0.36 Hz to 0.92 ± 0.21 Hz or by (29.1 ± 13.2)%.The STOC amplitudes were also decreased by XeC, from baseline 23.99 ± 2.90 pA to 17.49 ± 2.05 pA (percent decrease 24.4 ± 7.4%) after XeC application ($P < 0.05$, $n = 7$) (Fig. 1A). These results suggest that roughly 30% of the STOC are XeC-sensitive under physiological conditions.

We also examined the effect of exogenous IP3 on the STOC using regular whole-cell recording, in which the IP3 was added into the pipette solution and diffused into intracellular space. IP3 at concentrations 0.05, 0.5, 5 and 50 μM were applied in the pipette solution to observe the concentration-dependence of IP3 action. The results showed that 0.05 μM IP3 could not change the STOC, but IP3 at higher concentrations (0.5, 5 and 50 μM) all significantly increased the STOC and prolonged its persistence time (typical whole-cell recoding in Supplementary Fig. S1C and statistic data in Fig. 1B and C). IP3 blocker XeC (10 μM) applied in bath solution largely (about 50%) abolished the IP3-enhanced STOC (Fig. 1A). The STOC frequency was reduced by XeC from baseline 0.91 ± 0.24 Hz to 0.38 ± 0.16 Hz, and the STOC amplitude was reduced from 24.9 ± 3.8 pA to 14.08 ± 5.20 pA (Fig. 1A) ($P < 0.05$, $n = 4$). These results indicate that introduction of IP3 into the cytoplasm significantly increased the STOC at least partially via IP3R. We also noticed that 0.5, 5 or 50 μM IP3 presented similar effect on the STOC, suggesting that 0.5 μM IP3 can already exert a maximal effect on the STOC. Roughly 50% of the STOC are XeC-sensitive and therefore the activation of STOC is partially IP3R-dependent under experimental conditions above.

3.2. IP3 increases the macroscopic component of BK_{Ca} current via an IP3R-independent mechanism in porcine coronary smooth muscle cells

Interestingly, we found that the MC showed differential responses to blockades of IP3R and BK_{Ca} channel compared with that of STOC. Blocking IP3R by XeC did not appreciably affect the MC (Supplementary Fig. S2 and Fig. 2A). Induction of 50 μM IP3 into the cytoplasm induced a greater MC density than that by IP3 at lower concentrations (0.05, 0.5 and 5 μM) (Fig. 2B). These MC responses to IP3 were quite different from that of STOC. In the latter, IP3 at 0.5 μM already exerted a maximal (saturated) effect on the STOC (Fig. 1B and C). In order to demonstrate whether the IP3 action on MC shows a sarcoplasmic Ca^{2+}-dependent manner, we further observed the influence of thapsigargin (1 μM) (a depletor of SR Ca^{2+} store) on the MC action of IP3. Fig. 2C shows a clear result that 50 μM IP3 significantly increased the MC density even after SR Ca^{2+} depletion. These results suggest a different response pattern of MC to IP3 compared with that of STOC to IP3. Possibly, IP3 may use differential signaling pathways to enhance different components of BK_{Ca} currents, i.e., to activate the STOC via an IP3R-dependent pathway, and to stimulate the MC via both IP3R-dependent and independent pathways. One possibility is that high level IP3 may activate BK_{Ca} channel by affecting the Ca^{2+} sensitivity-related channel kinetics directly.

3.3. IP3 modulates the gating kinetics of BK_{Ca} channels in porcine coronary smooth muscle cells

To determine if IP3 can directly activate BK_{Ca} channel without signaling via IP3R, we examined the effect of IP3 on the single BK_{Ca} channel current using an inside-out patch clamp setting, by which the involvement of sarcoplasmic Ca^{2+} can be ruled out. The current properties of single BK_{Ca} channel at the inside-out configuration were the same as we previously reported [2]. At a symmetrical K^+ concentration (140 mM) at each side of the membrane, when the Ca^{2+} concentration was set at 0.1 μM at the cytoplasmic side of the patch, IP3 increased the BK_{Ca} current in a concentration (10–50 μM)-dependent manner (typical recording in Supplementary Fig. S3A). IP3 at lower concentration (<10 μM) could not significantly activate the single BK_{Ca} channel, which is consistent with the action of IP3 on the MC current (Fig. 2B). In addition, under this inside-out configuration, application of 1 μM IP3 in the bath solution did not significantly affect the NPo (baseline, 0.016 ± 0.004; 1 μM IP3, 0.018 ± 0.003) ($P > 0.05$, $n = 3$). Higher IP3 ($\geqslant$10 μM) significantly increased the NPo of BK_{Ca} channels, and this effect was saturated at IP3 concentrations up to 50 μM (Supplementary Fig. S3A and Fig. 3A). XeC did not alter the activating effect of IP3 on the single BK_{Ca} channel (Supplementary Fig. S3B).

In order to determine the specificity of the IP3 action, we tested the effect of D-myo-inositol-1, 3, 4, 5-tetraphosphate (IP4), an IP3 derivative which does not exert the specific effect on IP3R. IP4 at

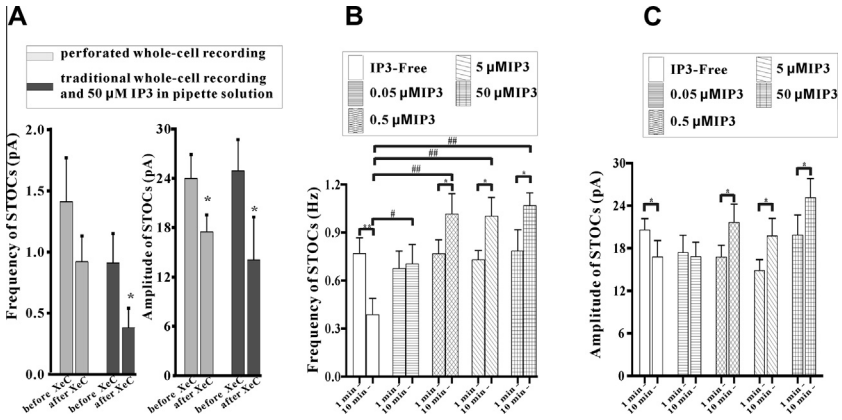

Fig. 1. Exogenous IP3 stimulated STOC generation. (A) inhibition of the frequency and amplitude of STOCs by XeC in perforated and traditional (regular) whole-cell recordings, showing that exogenous IP3 stimulated STOC generation. (B) and (C) summary bar graphs showing the enhancement of the STOC frequency and amplitude by 0.5, 5 and 50 μM IP3, but not by 0.05 μM IP3, compared with that at IP3-free condition. Data were obtained at 1 min and 10 min after the establishment of whole cell conformation (membrane rupture). $^{*}P < 0.05$ *vs.* self-control; $^{\#}P < 0.05$ *vs.* IP3-free group.

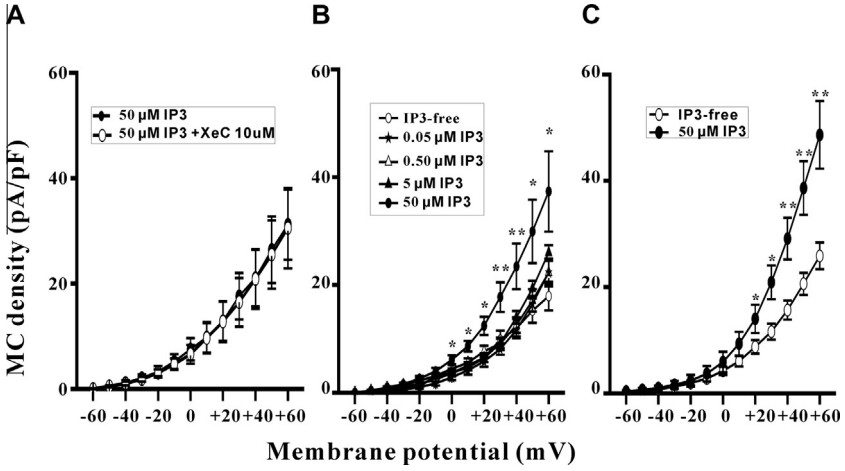

Fig. 2. The effect of IP3 on macroscopic currents (MC). (A) statistical data showing that XeC did not inhibit the MC. (B) *I–V* curves of MC 10 min after membrane rupture, showing that 50 μM IP3 stimulated the MC significantly. IP3-free, $n = 10$ (cells); IP3 0.05 μM, $n = 5$; IP3 0.5 μM, $n = 19$; IP3 5 μM, $n = 10$; IP3 50 μM, $n = 12$. (C) *I–V* curves of BK_{Ca} channels after treatment with 1 μM thapsigargin. Thapsigargin did not affect the action of IP3 on BK_{Ca} channel. IP3-free, $n = 7$; IP3 50 μM, $n = 8$. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* IP3-free at the same membrane potential.

very high concentration (50 μM) only slightly activated the BK_{Ca} currents (Supplementary Fig. S3C), and the *N*Po of BK_{Ca} channels increased only from baseline 0.141 ± 0.056 to 0.190 ± 0.096 ($n = 3$). By comparison, subsequent adding of IP3 remarkably increased the activity of single BK_{Ca} channel (Supplementary Fig. S3C), and the *N*Po of BK_{Ca} channel increased from baseline 0.190 ± 0.096 to 0.47 ± 0.197 ($n = 3$). The statistical data showed that IP3 concentration-dependently increased the *N*Po and decreased the mean close time, but did not affect the amplitude and mean open time of BK_{Ca} channels (Fig. 3A), suggesting that IP3 increases the open probability of BK_{Ca} channel and thus activates the channel mainly via decreasing the mean close time.

We further investigated the effect of IP3 on the kinetics of BK_{Ca} channels at different Ca^{2+} concentrations (0.5 and 1.0 μM) at the cytoplasmic side ($[Ca^{2+}]_i$). The two-order exponential curves were plotted to figure out the relationship between the open channel events and the dwell time, and to obtain the open time constants (τ_{O1}, τ_{O2}) and close time constants (τ_{C1}, τ_{C2}). The statistical results (Fig. 3B and C) indicate that 0.5 μM IP3 shifted the close time constant τ_{C2} from baseline 455.51 ± 119.70 ms ($n = 8$) to 57.10 ± 38.56 ms at 0.5 μM $[Ca^{2+}]$ i ($n = 4$, $P < 0.05$ *vs.* the IP3-free control), and 10 μM IP3 shifted the τ_{C2} to 33.77 ± 10.79 ms ($n = 4$, $P < 0.05$). IP3 had no effect on the close time constant τ_{C1} and the open time constants τ_{O1} and τ_{O2}, suggesting that IP3 increases the transition rate constant by decreasing τ_{C2} and modulates the channel from the closed state to the open state. In addition, as shown in Fig. 3B and C, IP3 showed similar effects at 0.5 and 1.0 μM $[Ca^{2+}]_i$. This effect of IP3 on τ_{C2} was similar to the effect of Ca^{2+} elevation: elevating the Ca^{2+} level at the cytoplasmic side increased the rate constant from the closed state to the open state, i.e., an elevation of Ca^{2+} level from 0.5 to 1.0 μM decreased the τ_{C2} from 455.51 ± 119.70 ms to 125.36 ± 38.56 ms ($n = 5$, $P < 0.05$). However, Ca^{2+} elevation also increased the open time constant τ_{O1} from 0.89 ± 0.14 ms to 1.43 ± 0.11 ms ($n = 5$, $P < 0.05$), suggesting that IP3 and Ca^{2+} shared similar actions but also exerted their own effects on channel kinetics. IP3 only affected the close time constant, while Ca^{2+} had influences on both the open and close time constants, indicating that the signaling pathways underlying the effects of IP3 and Ca^{2+} on BK_{Ca} channel are different.

3.4. IP3 stimulates BK_{Ca} currents via increasing the Ca^{2+} sensitivity of the channel

To address the potential mechanisms by which IP3 affected the BK_{Ca} channel kinetics, we evaluated the effect of IP3 on the Ca^{2+} sensitivity of BK_{Ca} channels. A freshly prepared IP3 solution was

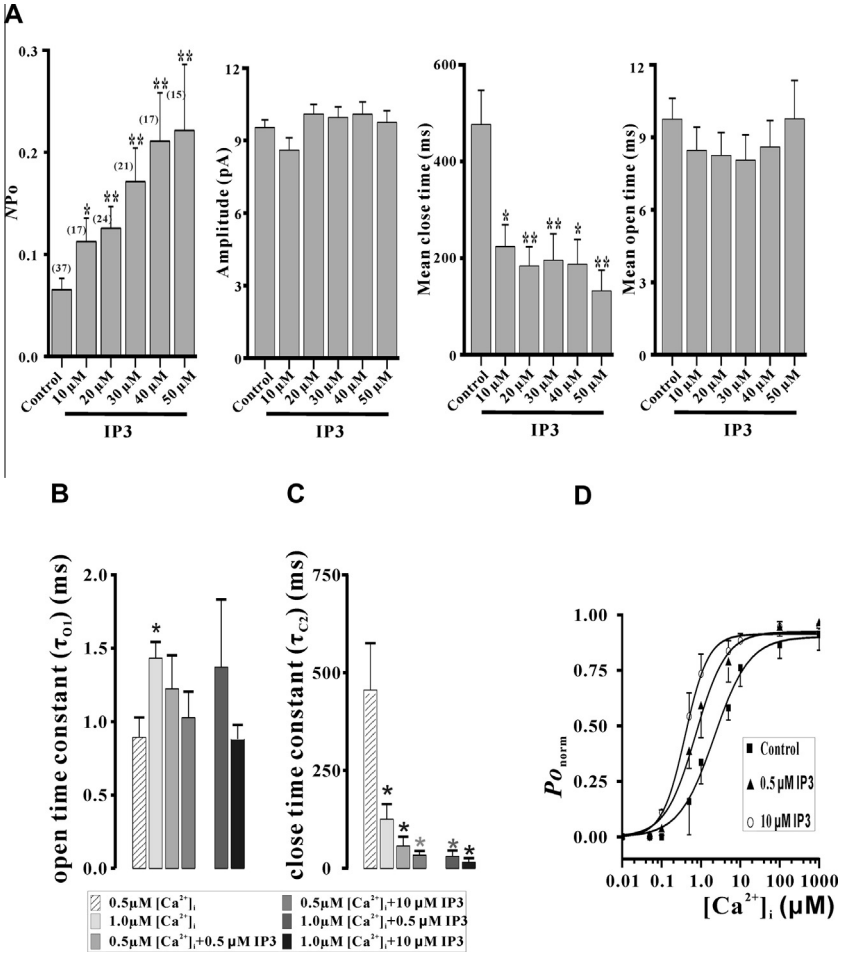

Fig. 3. The effect of IP3 on single BK_{Ca} currents in inside-out patches. (A) Bar graph summarizing the effects of IP3 on the kinetics of BK_{Ca} channels, including the open probability, mean close time, mean open time and current amplitude. $^{*}P < 0.05$ *vs.* control. Membrane potential was set to +40 mV. (B) and (C) statistical data showing the effect of IP3 on the gating kinetics of BK_{Ca} channels. Elevation of $[Ca^{2+}]_i$ but not IP3 significantly increased the open time constant, but elevation of $[Ca^{2+}]_i$ and exogenous IP3 (0.5 and 10 μM) both significantly decreased the close time constant of the channel ($n = 4$). $^{*}P <$ *vs.* 0.05 μM $[Ca^{2+}]_i$. (D) the dose-response curves representing fits to the Hill function ($Po = [Ca^{2+}]^n/(k^n+[Ca^{2+}]^n)$) in different treatments at the +40 mV membrane potential. Data were obtained from 4−5 cells. IP3 shifted the curves leftward, indicating an increase in the sensitivity of the channel to Ca^{2+}.

added to the bath solution to obtain a final concentration of 0.5 or 10 μM. Single-channel currents of inside-out patches were then recorded at membrane potentials ranging from −80 to + 80 mV, and at clamped $[Ca^{2+}]_i$ levels ranging from 10 nM to 10 mM. Data were analyzed at the membrane potential of +40 mV, where the openings of BK_{Ca} channels can be easily distinguished from other channels and more accurate statistical analysis can be performed. Typical current traces of single BK_{Ca} channel recorded at +40 mV are shown in Supplementary Fig. S3D, the plots of the normalized *Po vs.* $[Ca^{2+}]_i$ and the Hill fits are shown in Fig. 3D. The dose-response curve was shifted leftward by IP3. The corresponding $[Ca^{2+}]_i$ for half-maximal *Po* (EC_{50}, i.e., Michael constant *K*) were shifted from 5.02 ± 2.33 to 1.60 ± 0.94 μM with 0.5 μM IP3 to 0.70 ± 0.24 μM with 10 μM IP3, whereas the Hill constant was shifted from 1.89 ± 0.54 to 1.99 ± 0.39 at 0.5 μM IP3 to 2.26 ± 0.88 at 10 μM IP3. These results suggest that IP3 increases the sensitivity of BK_{Ca} channel to Ca^{2+}.

3.5. IP3 decreases coronary artery tone by activating IP3R and BK_{Ca} channels in coronary artery smooth muscles

This part of experiments aimed to determine if activation of BK_{Ca} channels by IP_3 has a physiological role, i.e., relaxing the coronary artery tone. As IP3 cannot penetrate the plasma membrane, it is difficult to directly observe the effect of exogenous IP3 on coronary artery tone. We therefore used blockers of IP3R and BK_{Ca} channel to indirectly estimate the effect of IP3 in this scenario. To observe the vaso-relaxing effects of these agents, the endothelium-denuded LAD rings were pre-constricted with 0.1 μM $PGF_{2\alpha}$. The LAD ring reactivity was maintained well as tested by 80 mM KCl after the experiments (Fig. 4). Under the present experimental condition, the $PGF_{2\alpha}$-induced tension increase was 49.7 ± 13.3% ($n = 11$) of that by 80 mM KCl. After $PGF_{2\alpha}$-induced LAD constriction reached its plateau, the use of XeC (2 μM), a selective IP3R blocker, induced a further increase of LAD tension by 26.10 ± 5.73% ($n = 4$) of that induced by $PGF_{2\alpha}$ (typical recording in Fig. 4A). Pretreatment of the coronary rings with 200 nM IbTX (a selective BK_{Ca} channel blocker) abolished the coronary artery constricting effect of XeC (Fig. 4B) ($n = 3$). Similar results were obtained with 2-APB (40 μM), an alternative IP3R blocker (Fig. 4C and D). Application of 40 μM 2-APB to the bath solution induced an further increase of coronary artery tension by 52.09 ± 19.64% ($n = 7$) of that induced by PGF2α. Given the evidence that IbTX induced a further constriction following $PGF_{2\alpha}$, and IbTX abolished the coronary artery constricting effects of IP3R blockers XeC and 2-APB (Fig. 4B and D), it is suggested that

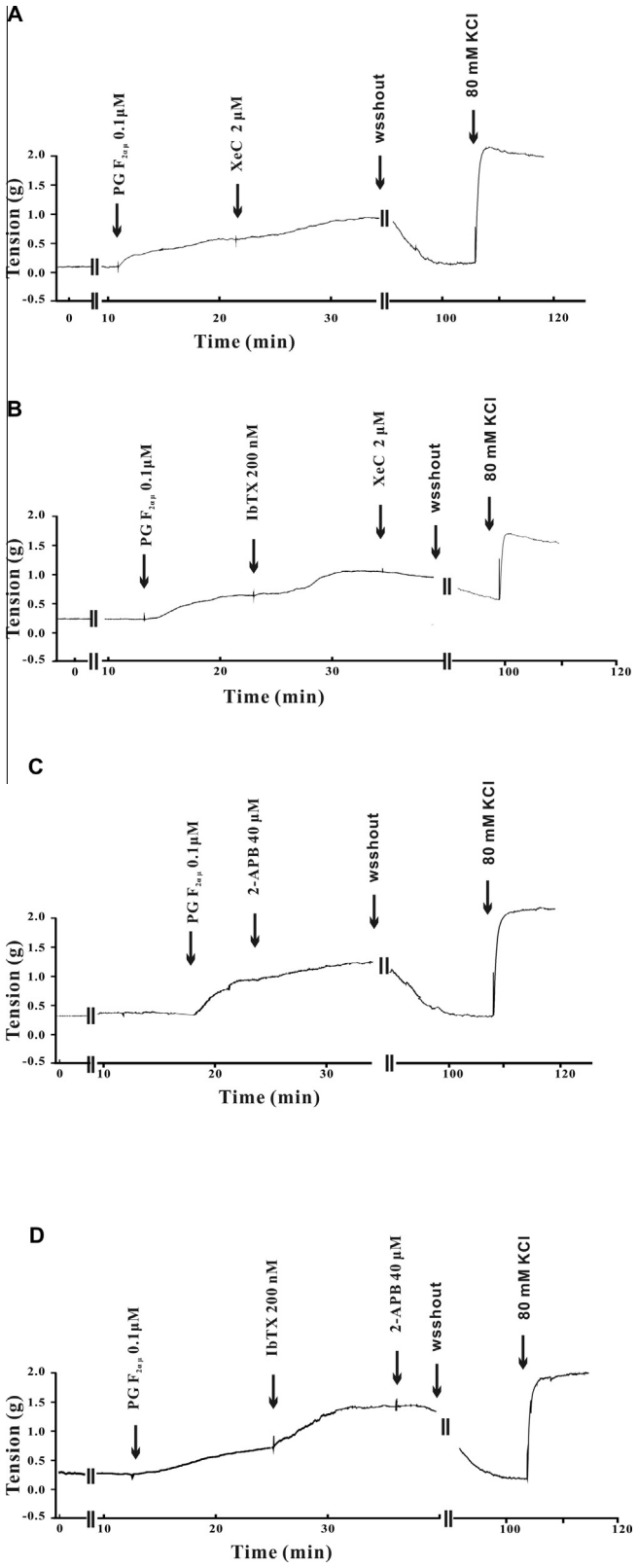

Fig. 4. Effects of XeC, 2-APB and IbTX on the tension of porcine coronary artery rings precontracted with $PGF_{2\alpha}$. (A) XeC (IP3R blocker) caused a marked tension increase ($n = 4$). (B) pretreatment of the coronary rings with 200 nM IbTX (BK_{Ca} channel blocker) abolished the effect of XeC ($n = 3$). (C) and (D) 2-APB (non-selective IP3R blocker) exerted similar effect as XeC ($n = 7$).

endogenous IP3 mediates coronary relaxation firstly via activating IP3R-Ca^{2+} signaling and then agonizing the BK_{Ca} channels.

4. Discussion

The hydrolysis of membrane-associated PIP2 to water-soluble IP3 is a common response in many cell types to a wide variety of external stimuli. IP3 works as a second messenger that increases intracellular Ca^{2+} by mobilizing internal Ca^{2+} stores. The elevation of IP3 level in arterial SMCs may affect the artery tone in two directions, i.e. increasing the artery tone via the Ca^{2+} mobilization-calmodulin-myosin light chain kinase (MLCK) pathway, or decreasing the artery tone via the BK_{Ca} channel-hyperpolarization pathway, depending on the stimuli, the post-receptor signaling scenario, and the vessel type. Because BK_{Ca} channel activation may induce coronary artery relaxation [4,5], pharmacological agents that stimulate BK_{Ca} channel activity may potentially be developed as coronary vasodilators, which is supported by the present study. However, to our knowledge, the regulatory mechanisms of BK_{Ca} channel activity in coronary artery are rarely investigated. Whether IP3 serves as a BK_{Ca} channel opener in the coronary SMCs is unclear. It is known that prostaglandins (PG) are important in the induction of coronary artery constriction, and this represents the integrated output of several complex signal transduction cascades that converge on the myofilaments and contractile apparatus. PGF2α could enhance oxidative stress in coronary heart disease and chronic heart failure [11,12]. Based on these findings, the present study used $PGF_{2\alpha}$ to preconstrict LAD rings in an attempt to determine if IP3 can serve as a coronary dilator by activating BK_{Ca} channels. We first demonstrated that IP3 activates BK_{Ca} channels in porcine coronary artery SMCs via two signaling pathways. One is that IP3 stimulates the IP3R-Ca^{2+} signaling and therefore by which activates BK_{Ca} channels; the other is that IP3 affects the channel gating kinetics from the cytoplasmic side by increasing the Ca^{2+} sensitivity of BK_{Ca} channels, and as a result, increases the open probability of the channel. We then demonstrated that IP3 could relax the coronary artery via activating IP3R and BK_{Ca} channels.

Local Ca^{2+} release (Ca^{2+} sparks) through ryanodine-sensitive and IP3-sensitive channels located in the SR of VSMCs activates nearby BK_{Ca} channels to generate STOCs that can be detected by whole-cell voltage clamp at the membrane potentials of physiological levels[13,14]. The present study confirmed that IP3 had a physiological contribution to STOC generation (approximately 30% of the STOCs are XeC-sensitive, and the remaining STOC might be ryanodine receptor-dependent which was not studied). Introduction of exogenous IP3 into the cytoplasm increased the XeC-sensitive STOCs roughly by 30–50% (Fig. 2C), indicating that IP3R-Ca^{2+} signaling pathway is involved in IP3-induced STOC generation, which may serve as an important mechanism for coronary tone regulation. These results are consistent with other study [15], in which Ca^{2+} sparks were achieved by dialyzing IP3 into the airway SMCs through the patch pipette solution, and dialysis of 10 μM IP3 significantly increased Ca^{2+} sparks.

Similar to our observation that IP3 activates BK_{Ca} channels from the cytoplasmic side, Zhao et al., [8] recently reported that IP3 activated BK_{Ca} channels both in intact cells and in excised inside-out membrane patches in rat and mouse VSMCs of cerebral arteries. The authors suggest that activation of IP3R1 elevates the Ca^{2+} sensitivity of BK_{Ca} channel through local molecular coupling in these VSMCs. Another report [16] showed that IP3R-mediated vascular relaxation was elicited through cyclic adenosine monophosphate (cAMP)-dependent and independent routes and both routes included BK_{Ca} channel activation. It was further showed that the cAMP-independent vasorelactant mechanism is partially due to direct activation of BK_{Ca} channels by G(s)-protein. Some other studies suggest the direct regulation of BK_{Ca} channels by other endogenous molecules. For example, BK_{Ca} channels are thought

to be regulated only by phospholipase C (PLC)-generated PIP_2 metabolites that target Ca^{2+} stores and protein kinase C (PKC), and eventually BK_{Ca} channels [17]. The authors reported that PIP2 activates BK_{Ca} channels in a PIP2 metabolites-independent manner. PIP2 enhances Ca^{2+}-driven gating and alters both the opening and closed channel distributions without affecting the voltage gating and unitary conductance. This is partially consistent with our inside-out patch results that IP3 enhanced the *N*Po and altered the close time of the BK_{Ca} channel without affecting the unitary conductance. We also showed that IP3 altered the kinetic properties of BK_{Ca} channels by increasing the Ca^{2+} sensitivity of the channel at a wide range of membrane potentials, potentially contributing to the enhancement of BK_{Ca} channel activity.

In order to exclude the possibility that the effect of higher IP3 on the MC of BK_{Ca} currents is dependent on intracellular Ca^{2+} stores, we pretreated the SMCs with TG (a SR Ca^{2+} depletor) and found that higher IP3 (50 μM) could still induce large MC at the presence of TG (Fig. 2C). To exclude the possibility that BK_{Ca} channel-associated IP3Rs may exist in the inside-out patch, we examined the effect of XeC on IP3 action in this configuration. The results showed that both the enhancement of MC under regular whole-cell configuration and the increase of single BK_{Ca} channel current under inside-out patch configuration by higher IP3 were IP3R-independent. We also tested the possibility of the non-specific effect of IP3 in inside-out patches at higher concentration. The results showed that IP4, an IP3 analogue that does not activate IP3R, had minor effect on BKCa channels at high concentration, similar to the effect of IP3 at high concentration. However, IP3 does show a specific effect on BK_{Ca} channel at relatively lower dosage compared with IP4. Other possibilities, such as IP3 binds to a particular site of the BK_{Ca} channel (α or β subunit of BK_{Ca} channel, or other domains) and by which directly affects the function of the channel, deserves further investigation.

In summary, the present study provides evidence to support that IP3 can relax coronary arteries via activating the BK_{Ca} channel. This action of IP3 is likely mediated by two signaling pathways, i.e., exciting the IP3R or interacting directly with the BK_{Ca} channel. Since the activation of vascular BK_{Ca} channels acts as an important feedback mechanism that opposes excitation and limits contraction, increased activity of BK_{Ca} channels may have a large impact to prevent vasospasm of coronary vasculature. The coordination of the two pathways of IP3-induced BK_{Ca} activation may play an important role in the functional regulation of BK_{Ca} channels and therefore may imply a new mechanism for coronary artery relaxation. Strategies that elevate IP3 level and/or activate BK_{Ca} channel of coronary artery SMCs may have potential clinical perspectives in the development of new coronary artery relaxing drugs.

Acknowledgments

This work was supported by grants from the National Natural Science Foundation of China (30370527 to Zeng XR; 30670763 and 81173661 to Yang Y). We thank Dr. Cao JM and Dr. Zeng B for manuscript reading and comments.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.08.079.

References

[1] M. Gollasch, C. Ried, R. Bychkov, F.C. Luft, H. Haller, K+ currents in human coronary artery vascular smooth muscle cells, Circ. Res. 78 (1996) 676–688.
[2] Y. Yang, F. Cai, P.Y. Li, M.L. Li, J. Chen, G.L. Chen, Z.F. Liu, X.R. Zeng, Activation of high conductance Ca(2+)-activated K(+) channels by sodium tanshinoneII-A sulfonate (DS-201) in porcine coronary artery smooth muscle cells, Eur. J. Pharmacol. 598 (2008) 9–15.
[3] S. Hou, S.H. Heinemann, T. Hoshi, Modulation of BKCa channel gating by endogenous signaling molecules, Physiology 24 (2009) 26–35.
[4] R.E. White, D.J. Darkow, J.L. Lang, Estrogen relaxes coronary arteries by opening BKCa channels through a cGMP-dependent mechanism, Circ. Res. 77 (1995) 936–942.
[5] J.L. Balwierczak, C.M. Krulan, H.S. Kim, D. DelGrande, G.B. Weiss, S. Hu, Evidence that BKCa channel activation contributes to K+ channel opener induced relaxation of the porcine coronary artery, Naunyn Schmiedebergs Arch. Pharmacol. 352 (1995) 213–221.
[6] V.K. Gribkoff, J.E. Starrett Jr., S.I. Dworetzky, P. Hewawasam, C.G. Boissard, D.A. Cook, S.W. Frantz, K. Heman, J.R. Hibbard, K. Huston, G. Johnson, B.S. Krishnan, G.G. Kinney, L.A. Lombardo, N.A. Meanwell, P.B. Molinoff, R.A. Myers, S.L. Moon, A. Ortiz, L. Pajor, R.L. Pieschl, D.J. Post-Munson, L.J. Signor, N. Srinivas, M.T. Taber, G. Thalody, J.T. Trojnacki, H. Wiener, K. Yeleswaram, S.W. Yeola, Targeting acute ischemic stroke with a calcium-sensitive opener of maxi-K potassium channels, Nat. Med. 7 (2001) 471–477.
[7] E. Moczydlowski, O. Alvarez, C. Vergara, R. Latorre, Effect of phospholipid surface charge on the conductance and gating of a Ca2+-activated K+ channel in planar lipid bilayers, J. Membr. Biol. 83 (1985) 273–282.
[8] G. Zhao, Z.P. Neeb, M.D. Leo, J. Pachuau, A. Adebiyi, K. Ouyang, J. Chen, J.H. Jaggar, Type 1 IP3 receptors activate BKCa channels via local molecular coupling in arterial smooth muscle cells, J. Gen. Physiol. 136 (2010) 283–291.
[9] D.R. Bell, R.C. Webb, D.F. Bohr, Functional bases for individualities among vascular smooth muscles, J. Cardiovasc. Pharmacol. 7 Suppl 3 (1985) S1–S11.
[10] Y. Yang, P.Y. Li, J. Chen, L. Mao, J. Wen, X.Q. Tan, Z.F. Liu, X.R. Zeng, Function of BKCa channels is reduced in human vascular smooth muscle cells from Han Chinese patients with hypertension, Hypertension 61 (2013) 519–525.
[11] Z. Mallat, I. Philip, M. Lebret, D. Chatel, J. Maclouf, A. Tedgui, Elevated levels of 8-iso-prostaglandin F2alpha in pericardial fluid of patients with heart failure: a potential role for in vivo oxidant stress in ventricular dilatation and progression to heart failure, Circulation 97 (1998) 1536–1539.
[12] R. Wolfram, A. Oguogho, B. Palumbo, H. Sinzinger, Enhanced oxidative stress in coronary heart disease and chronic heart failure as indicated by an increased 8-epi-PGF(2alpha), Eur. J. Heart Fail. 7 (2005) 167–172.
[13] M.T. Nelson, H. Cheng, M. Rubart, L.F. Santana, A.D. Bonev, H.J. Knot, W.J. Lederer, Relaxation of arterial smooth muscle by calcium sparks, Science 270 (1995) 633–637.
[14] P. Li, X. Zeng, Y. Yang, F. Cai, Z. Liu, M. Li, J. Pei, W. Zhou, Role of calcium mobilization in the regulation of spontaneous transient outward currents in porcine coronary artery myocytes, Sci. China C Life Sci. 50 (2007) 660–668.
[15] Q.H. Liu, Y.M. Zheng, Y.X. Wang, Two distinct signaling pathways for regulation of spontaneous local Ca2+ release by phospholipase C in airway smooth muscle cells, Pflugers Arch. 453 (2007) 531–541.
[16] Y. Tanaka, F. Yamaki, K. Koike, L. Toro, New insights into the intracellular mechanisms by which PGI2 analogues elicit vascular relaxation: cyclic AMP-independent, Gs-protein mediated-activation of MaxiK channel, Curr. Med. Chem. Cardiovasc. Hematol. Agents 2 (2004) 257–265.
[17] T. Vaithianathan, A. Bukiya, J. Liu, P. Liu, M. Asuncion-Chin, Z. Fan, A. Dopico, Direct regulation of BK channels by phosphatidylinositol 4,5-bisphosphate as a novel signaling pathway, J. Gen. Physiol. 132 (2008) 13–28.