Constitutive PKA activity is essential for maintaining the excitability and contractility in guinea pig urinary bladder smooth muscle: role of the BK channel

Wenkuan Xin,1* Ning Li,1,2* Qiuping Cheng,1 Vitor S. Fernandes,1 and Georgi V. Petkov1

1Department of Drug Discovery and Biomedical Sciences, South Carolina College of Pharmacy, University of South Carolina, Columbia, South Carolina; and 2Department of Urology, Fourth Hospital of China Medical University, Shenyang, China

Submitted 2 June 2014; accepted in final form 8 October 2014

Xin W, Li N, Cheng Q, Fernandes VS, Petkov GV. Constitutive PKA activity is essential for maintaining the excitability and contractility in guinea pig urinary bladder smooth muscle: role of the BK channel. Am J Physiol Cell Physiol 307: C1142–C1150, 2014. First published October 15, 2014; doi:10.1152/ajpcell.00167.2014—The elevation of protein kinase A (PKA) activity activates the large-conductance voltage- and Ca2+-activated K+ (BK) channels in urinary bladder smooth muscle (UBSM) cells and consequently attenuates spontaneous phasic contractions of UBSM. However, the role of constitutive PKA activity in UBSM function has not been studied. Here, we tested the hypothesis that constitutive PKA activity is essential for controlling the excitability and contractility of UBSM. We used patch clamp electrophysiology, line-scanning confocal and ratiometric fluorescence microscopy on freshly isolated guinea pig UBSM cells, and isometric tension recordings on freshly isolated UBSM strips. Pharmacological inhibition of the constitutive PKA activity with H-89 or PKI 14–22 significantly reduced the frequency and amplitude of spontaneous transient BK channel currents (TBKCs) in UBSM cells. Confocal and ratiometric fluorescence microscopy studies revealed that inhibition of constitutive PKA activity with H-89 reduced the frequency and amplitude of the localized Ca2+ sparks but increased global Ca2+ levels and the magnitude of Ca2+ oscillations in UBSM cells. H-89 abolished the spontaneous transient membrane hyperpolarizations and depolarized the membrane potential in UBSM cells. Inhibition of PKA with H-89 or KT-5720 also increased the amplitude and muscle force of UBSM spontaneous phasic contractions. This study reveals the novel concept that constitutive PKA activity is essential for controlling localized Ca2+ signals generated by intracellular Ca2+ stores and cytosolic Ca2+ levels. Furthermore, constitutive PKA activity is critical for mediating the spontaneous TBKCs in UBSM cells, where it plays a key role in regulating spontaneous phasic contractions in UBSM.

Constictive PKA activity increases SR Ca2+-ATPase activity and SR Ca2+ release signaling (2, 22, 32, 41). Notably, the elevation of PKA activity leads to an increase in the rapid and localized Ca2+ releases from the SR via ryanodine receptors (RyRs) known as “Ca2+ sparks” and consequently increases transient BK channel currents (TBKCs) in UBSM cells (4, 19, 32, 39, 44). However, prior to the current study, the physiological role of constitutive PKA activity in the regulation of UBSM excitability and contractility was not clearly understood.

It has been demonstrated that elevated PKA activity plays an important role in β-adrenergic receptor activation as well as phosphodiesterase (PDE) inhibition-mediated suppression of UBSM excitability and contractility (4, 13, 31, 32, 39, 44). cAMP/PKA activity depends on the balance between the activity of adenylyl cyclases, which convert ATP to cAMP, and of PDEs that hydrolyze cAMP to AMP (9). The activation of adenylyl cyclases via β-adrenergic receptors or pharmacological inhibition of PDEs shifts the balance, leading to a rapid increase in cellular cAMP levels and PKA activity (9). The elevation of PKA activity can effectively increase TBKCs in UBSM cells and attenuate both the excitability and spontaneous phasic contractions of UBSM (4, 19, 44). Specifically, the elevation of PKA activity as a result of β-adrenergic receptor activation or PDE4 inhibition increases Ca2+ spark activity and the functionally coupled TBKCs, resulting in suppression of the excitability and spontaneous phasic contractions of guinea pig UBSM (32, 44). In addition, the increase in PKA expression compensates for the loss of BK channels in BK channel knockout mice and enhances the β-adrenergic receptor-mediated attenuation of UBSM contractions (4, 39).

Specifically, the elevation of cellular cAMP levels increases PKA phosphorylation of phospholamban (PLB), a regulatory protein of the Ca2+-ATPase. The phosphorylated PLB has a reduced inhibitory effect on Ca2+-ATPase compared with the dephosphorylated PLB (22). Consequently, the elevation of PKA activity increases SR Ca2+-ATPase activity and SR Ca2+ content (22). The elevated SR Ca2+ load increases the open probability of RyRs and the activity of Ca2+ sparks (12). However, whether constitutive PKA activity is necessary for the spontaneous Ca2+ sparks and TBKCs has yet to be investigated. The present study determined the physiological function of constitutive PKA activity in UBSM by investigating its regulation of basal Ca2+ sparks, TBKCs, spontaneous transient...
hyperlaminations in UBSM cells, and the spontaneous phasic contractions of the UBSM.

MATERIALS AND METHODS

UBSM tissue collection and single-cell isolation. A total of 31 male Hartley Albino guinea pigs (Charles River Laboratory, Raleigh, NC) with an average weight of 437 ± 11 g were used in this study. Guinea pigs were euthanized with CO2 inhalation, followed by thoracotomy, following animal use protocol no. 1747 that was reviewed and approved by the Institutional Animal Care and Use Committee of the University of South Carolina. UBSM strips (~2–3 mm wide and ~6–7 mm long) were prepared by removing the mucosa and used for single UBSM cell isolation and isotonic UBSM tension recordings. UBSM single cells were freshly isolated by enzymatic digestion of UBSM strips in solutions containing papain and then in solutions containing type II collagenase, following the procedures as described previously (43–45).

Electrophysiological recordings. The amphotericin B-perforated whole cell patch clamp technique was used for electrophysiological recordings in freshly isolated UBSM single cells, as described previously (43–45). The TBKCs were recorded at a holding potential of −40 mV, using a pipette with a resistance between 4 and 6 MΩ. The membrane potential of UBSM cells was recorded in current clamp mode of the patch clamp technique (Ih = 0). All patch clamp experiments were conducted at room temperature (~22–23°C).

Ca²⁺ spark recordings with high-speed line-scanning confocal microscopy in freshly isolated UBSM cells. Ca²⁺ sparks were recorded in cells loaded with the fluorescent Ca²⁺ probe fluo 4-AM using a high-speed line-scanning LSM700-Meta confocal microscope and analyzed using ImageJ with the SparkMaster plug-in, as reported previously (33, 44). The threshold for the detection of events was 3.8 times the standard deviation of the background noise over the mean value of the background intensity. The Ca²⁺ spark frequency and amplitude were averaged for the last five scanning cycles under control or test compound-treated conditions, respectively.

Recording of global intracellular Ca²⁺ levels in freshly isolated UBSM cells. The global intracellular Ca²⁺ levels were monitored using a ratiometric fluorescent Ca²⁺ probe fura 2-AM, as described previously (45). Cells were imaged with an Olympus IX81 inverted microscope equipped with a ×40 oil objective and MetaFluor 7.7.2.0 software (Molecular Devices, Sunnyvale, CA). Fura 2 was excited for 20 ms at 340 and 380 nm of light with an interval of 0.6 s. The relative intracellular Ca²⁺ level was expressed as the ratio of F₃₄₀/F₃₈₀ emission intensities at 510 nm. All Ca²⁺ imaging experiments were carried out at room temperature (22–23°C).

Isometric UBSM tension recordings. Isometric contractions of UBSM isolated strips were measured using a Myomed myograph system (MED Associates, St. Albans, VT), as described previously (43–45). To minimize the potential effects of neurotransmitter release from neurons in the UBSM tissue, the spontaneous phasic contractions were recorded in the presence of 1 μM tetrodotoxin, a selective blocker of the neuronal Na⁺ channels.

Data analysis and statistics. The amplitude and frequency of TBKCs, in addition to the UBSM phasic contraction parameters, were analyzed using MiniAnalysis (Synaptosoft, Decatur, GA). The cell membrane potential was analyzed using Clampfit 10.2 (Molecular Devices, Union City, CA). The last 5 min of recording under control or treated conditions were analyzed. Data were further analyzed with GraphPad Prism 4.03 software (GraphPad Software, La Jolla, CA). For the statistical analysis, the parameters of Ca²⁺ sparks, TBKCs, and spontaneous contractions were normalized to their controls from the same cell or UBSM strip, respectively. Data are expressed as means ± SE; n = the number of cells or UBSM strips, and N = number of guinea pigs. Statistical significance was performed using Student’s t-test, and P < 0.05 was considered significant.

Solutions and drugs. The nominally Ca²⁺-free dissection solution (in mM) 80 monosodium glutamate, 55 NaCl, 6 KCl, 10 glucose, 10 HEPES, and 2 MgCl₂; pH was adjusted to 7.3 with NaOH. The extracellular solution for whole cell patch clamp and Ca²⁺ imaging experiments contained (in mM) 134 NaCl, 6 KCl, 1 MgCl₂,
2 CaCl$_2$, 10 glucose, and 10 HEPES; pH was adjusted to 7.4 with NaOH. The pipet solution contained (in mM) 110 potassium aspartate, 30 KCl, 10 NaCl, 1 MgCl$_2$, 10 HEPES, and 0.05 EGTA; pH was adjusted to 7.2 with NaOH and supplemented with freshly dissolved (every 1–2 h) 200 μg/ml amphotericin B. The Ca$^{2+}$-containing physiological saline solution was prepared daily and contained (in mM) 119 NaCl, 4.7 KCl, 24 NaHCO$_3$, 1.2 KH$_2$PO$_4$, 2.5 CaCl$_2$, 1.2 MgSO$_4$, and 11 glucose and was aerated with 95% O$_2$-5% CO$_2$ to obtain pH 7.4. Myristoylated PKI 14–22 (Myr-N-Gly-Arg-Thr-Gly-Arg-Arg-Asn-Ala-Ile-NH$_2$), trypsin inhibitor, BSA, and amphotericin B were obtained from Thermo Fisher Scientific (Fair Lawn, NJ). Papain was purchased from Worthington Biochemical (Lakewood, NJ). N-[2-[3-(4-bromophenyl)-2-propenylamino]ethyl]-5-isooquinolinesulfonamide dihydrochloride (H-89), DT-2 trifluoroacetate salt (Tyr-Gly-Arg-Lys-Lys-Arg-Arg-Arg-Arg-Pro-Pro-Leu-Arg-Lys-lys-Lys-Lys-His-NH$_2$), collagenase (type II), and tetrodotoxin (in citrate buffer) were purchased from Sigma-Aldrich (St. Louis, MO). KT-5720 {[(9R,10S,12S)-2,3,9,10,11,12-hexahydro-10-hydroxy-9-methyl-1-oxo-9,12-epoxy-1H-diindolo[1,2,3-fg:3,2,1-hkl]pyrrolo[3,4-i][1,6]benzo-diazocine-10-carboxylic acid, hexyl ester} was purchased from R&D Systems (Minneapolis, MN). Amphotericin B, H-89, and KT-5720 were dissolved in dimethyl sulfoxide (DMSO) as stock solutions. All other chemicals were dissolved in double-distilled water. The maximum DMSO concentration was <0.1%.

Fig. 2. Pharmacological inhibition of PKG with DT-2 did not have any effect on the TBKCs in freshly isolated UBSM cells. A: an original voltage clamp recording illustrating that DT-2 (1 μM) did not have a significant effect on the frequency (Freq) and amplitude (Amp) of TBKCs (n = 6, N = 5). P > 0.05.

Fig. 3. Pharmacological inhibition of PKA with H-89 suppressed the basal level of Ca$^{2+}$ sparks in freshly isolated UBSM cells. A: an image of a freshly isolated UBSM cell loaded with fluo 4-AM. The white line passing through the active site $a$ is the laser beam-scanning pathway (1-pixel width). B: the 3-dimensional view of the recordings illustrate the Ca$^{2+}$ sparks in the absence and presence of H-89 (10 μM). The color scale indicates the relative fluorescence intensity F/F$_0$. C: summary data illustrating that H-89 (10 μM) decreased the Ca$^{2+}$ spark frequency (Freq) and amplitude (Amp) in UBSM cells (n = 8, N = 6) *P < 0.05.
RESULTS

Pharmacological inhibition of constitutive PKA activity reduced the spontaneous TBKCs in UBSM cells. Inhibition of PKA with H-89 (10 μM) led to an inhibition of spontaneous TBKCs in freshly isolated UBSM cells (Fig. 1). In two of the six tested cells, H-89 (10 μM) inhibited TBKCs, and in other four cells, H-89 completely abolished TBKCs. The summary data show that the inhibition of the constitutive PKA activity significantly reduced the frequency and amplitude of spontaneous TBKCs down to 5.4 ± 3.6 and 17.0 ± 11.6% of the control values, respectively (n = 6, N = 6, P < 0.05; Fig. 1).

To confirm that the effect of H-89 on TBKCs was due to the inhibition of constitutive PKA activity, we applied another highly selective and membrane-permeable peptide inhibitor of PKA, myristoylated PKI 14–22. PKI 14–22 (5 μM) inhibited TBKCs in UBSM cells (Fig. 1). The summary data show that PKI 14–22 decreased the frequency and amplitude of TBKCs down to 17.0 ± 7.4 and 52.6 ± 17.8% of the control, respectively (n = 6, N = 5, P < 0.05; Fig. 1). These data confirm that constitutive PKA activity is essential for the spontaneous TBKCs in UBSM cells.

The inhibitory effect of H-89 on TBKCs was due to the inhibition of PKA. To further clarify that the effect of H-89 on TBKCs was due to the inhibition of PKA and not inhibition of protein kinase G (PKG), we applied a PKG inhibitor prior to the addition of H-89. DT-2 (1 μM), a selective PKG inhibitor, alone did not affect TBKCs in freshly isolated UBSM cells (Fig. 2). The summary data show that, in the presence of DT-2, the frequency and amplitude of TBKCs were 104.4 ± 20.4 and 92.0 ± 10.9% of the control values, respectively (n = 6, N = 5, P > 0.05; Fig. 2). The subsequent addition of H-89 (10 μM) reduced the frequency and amplitude of TBKCs down to 2.7 and 13.8% of the control in one cell, respectively; H-89 completely abolished the TBKCs in the other three cells (n = 4, N = 4). These data support that PKA, but not PKG, played a role in the inhibition of TBKCs caused by H-89 (10 μM).

Pharmacological inhibition of constitutive PKA activity with H-89 decreased Ca²⁺ spark amplitude and frequency in freshly isolated UBSM cells. Ca²⁺ sparks transiently activate BK channels and generate TBKCs in UBSM cells (17, 32, 44). We used high-speed line-scanning confocal microscopy to measure Ca²⁺ sparks in freshly isolated UBSM cells. H-89 (10 μM) decreased the Ca²⁺ spark activity (Fig. 3). The summary data demonstrate that H-89 (10 μM) significantly decreased the Ca²⁺ spark frequency and amplitude down to 58.1 ± 13.9 and 79.7 ± 7.8% of the control values, respectively (n = 8, N = 6, P < 0.05; Fig. 3). These results demonstrate that the constitutively active PKA is critical for the generation of Ca²⁺ sparks in UBSM cells in the absence of external stimulation. The decrease in the Ca²⁺ spark activity most likely underlies the inhibition of the spontaneous TBKCs caused by PKA inhibitors.

Pharmacological inhibition of constitutive PKA activity with H-89 increased the global cytosolic Ca²⁺ levels in freshly isolated UBSM cells. The effect of constitutive PKA activity on global cytosolic Ca²⁺ levels in UBSM cells was investig-
Pharmacological inhibition of PKA with H-89 prevented caffeine-induced increase of TBKCs in freshly isolated UBSM cells. Caffeine is a potent RyR opener that increases the open probability of RyRs and SR Ca\textsuperscript{2+} release. Caffeine at a concentration >10 mM can deplete SR Ca\textsuperscript{2+} by releasing Ca\textsuperscript{2+} through RyRs (11). In UBSM cells, caffeine (10 mM) transiently increased TBKCs, followed by a complete inhibition (n = 9, N = 6; Fig. 5). However, when the constitutive PKA activity was inhibited with H-89 (10 μM), TBKCs were abolished, and the subsequent addition of caffeine (10 mM) did not induce any TBKCs (n = 5, N = 5; Fig. 5). These data suggest that inhibition of constitutive PKA activity might deplete SR Ca\textsuperscript{2+}, which is necessary for generating Ca\textsuperscript{2+} sparks and TBKCs.

Pharmacological inhibition of constitutive PKA activity with H-89 blocked the spontaneous transient hyperpolarizations and caused membrane depolarization in freshly isolated UBSM cells. Our previous study demonstrated that spontaneous TBKCs result in spontaneous transient hyperpolarizations in isolated UBSM cells (18, 45). Inhibition of the constitutive PKA activity with H-89 (10 μM) eliminated the spontaneous transient hyperpolarizations of the cell membrane and significantly depolarized the cell membrane potentials from −24.0 ± 3.3 mV under control conditions to −20.1 ± 2.5 mV in the presence of H-89 (n = 11, N = 8, P < 0.05; Fig. 6). These data suggest that constitutive PKA activity is necessary for the spontaneous transient hyperpolarizations in UBSM cells, and therefore, it is important in the regulation of UBSM excitability.

Pharmacological inhibition of constitutive PKA activity leads to an increase in the spontaneous phasic contractions of UBSM. Inhibition of PKA with H-89 increased the spontaneous phasic contractions of isolated UBSM strips (Fig. 7). The summary data show that the inhibition of PKA with H-89 (10 μM) significantly increased UBSM spontaneous phasic contraction amplitude, muscle force integral, and duration to 140.5 ± 16.8, 180.1 ± 25.9, and 127.2 ± 9.1% of the control, respectively (n = 14, N = 10, P < 0.05; Fig. 7). H-89 (10 μM) also significantly decreased the frequency of spontaneous phasic contractions to 70.6 ± 4.6% of the control (n = 14, N = 10, P < 0.05; Fig. 7). However, H-89 did not have any effect on UBSM tone, which was 93.1 ± 7.4% of the control (n = 14, N = 10, P > 0.05; Fig. 7).

To confirm that the effect of H-89 on the spontaneous phasic contractions of UBSM was indeed caused by the
inhibition of PKA, we used another selective PKA inhibitor, KT-5720. Figure 7 illustrates that KT-5720 (3 μM) increased the amplitude, muscle force integral, duration, and tone of the spontaneous phasic contractions to 168.4 ± 23.8, 214.0 ± 39.6, 124.8 ± 11.0, and 115.4 ± 6.6% of the control values, respectively (n = 8, N = 5, P < 0.05; Fig. 7). These data suggest that the constitutive PKA activity is important for regulating the spontaneous phasic contractions of the UBSM, as illustrated in Fig. 8.

DISCUSSION

We tested the hypothesis that constitutively active PKA is essential for the spontaneous TBKCs in UBSM cells and spontaneous phasic contractions of UBSM tissue. We provided direct evidence at the cellular and tissue levels that constitutive PKA activity controls spontaneous TBKCs by regulating their functionally coupled Ca^2+ sparks transiently activate BK channels, generating spikes in guinea pig UBSM cells.}

Fig. 7. Pharmacological inhibition of PKA with H-89 or KT-5720 significantly increased spontaneous phasic contractions of isolated UBSM strips. A and B: representative recordings of UBSM strips illustrating that pharmacological PKA inhibition increases UBSM spontaneous phasic contractions. C and D: summary data illustrating the effects of PKA inhibition with H-89 (10 μM; n = 14, N = 10) or KT-5720 (3 μM; n = 8, N = 5) on the amplitude (Amp), muscle force integral (Force), duration (Dur), frequency (Freq), and tone of UBSM spontaneous phasic contractions. *P < 0.05.
More importantly, this study is in line with our recent report, which showed that increased Ca\(^{2+}\) spark activity can occur simultaneously with a reduction of global Ca\(^{2+}\) levels (44).

Furthermore, when constitutive PKA activity was inhibited with H-89, caffeine (10 mM), a RyR opener, did not induce any TBKCs, supporting the hypothesis that the inhibition of constitutive PKA activity depletes SR Ca\(^{2+}\), resulting in attenuated Ca\(^{2+}\) spark activity (Fig. 5). Caffeine (10 mM) transiently increases TBKCs and is followed by a complete inhibition of TBKCs due to the increased release of Ca\(^{2+}\) and subsequent depletion of SR Ca\(^{2+}\) content (5, 47). The caffeine-induced inhibition of TBKCs has been used as an indication of the depletion of SR Ca\(^{2+}\) in smooth muscle cells (3, 41). Likewise, in UBSM cells, caffeine (10 mM) initially transiently increased but then completely abolished TBKCs (Fig. 5). When the constitutive PKA activity was inhibited and TBKCs were abolished, caffeine (10 mM) was no longer able to induce any TBKCs in UBSM cells (Fig. 5), suggesting that the inhibition of constitutive PKA activity can deplete SR Ca\(^{2+}\) prior to the application of caffeine. These results emphasize the essential role of constitutive PKA activity in maintaining SR Ca\(^{2+}\) content and the spontaneous TBKC activity.

Spontaneous TBKCs caused by basal Ca\(^{2+}\) spark activity regulate the excitability and spontaneous phasic contractions of UBSM (17, 23, 30, 32, 44). The spontaneous TBKCs and transient membrane hyperpolarizations are highly correlated when measured by voltage clamp and current clamp techniques in the same UBSM cells (18, 45). Inhibition of the constitutive PKA activity abolished the spontaneous transient hyperpolarizations and significantly depolarized the membrane potential of isolated UBSM cells (Fig. 6). The essential role of constitutive PKA activity in controlling UBSM excitability is supported by the observation that an increase in the expression of PKA compensates for the permanent loss of BK channels and the gain of excitability in UBSM upon genetic deletion of the BK channels (4, 39). These findings demonstrate that constitutive PKA activity is fundamentally important for the physiological functions of UBSM.

The major finding of the current study is that constitutive PKA activity is essential for the basal TBKCs in UBSM cells (Fig. 1), and this was confirmed by using the highly selective peptide PKA inhibitor PKI 14–22 in addition to H-89 (Fig. 1). It has been reported that H-89 has in vivo IC\(_{50}\) values ranging from 10 to 30 μM, depending on the intracellular ATP levels, and 10 μM H-89 has been used to inhibit PKA activity in most cell-based studies (6, 16). H-89 at low micromolar range may not be sufficient to inhibit endogenous PKA activity (10). In the present study, we used 10 μM H-89 to investigate the effects of constitutive PKA activity on TBKCs, which was further examined using a highly selective peptide inhibitor, PKI 14–22.

As for many ATP-competitive kinase inhibitors, the specificity and the nonkinase-dependent effects of H-89 have raised concerns in studies investigating the function of PKA (24, 26,
36). In the present study, we measured TBKCs, which are activated solely by the localized Ca\(^{2+}\) sparks in freshly isolated UBSM cells (8, 17). The Ca\(^{2+}\) spark activity decreased in the presence of H-89, leading to the reduction of TBKCs’ frequency and amplitude (Figs. 1 and 2). These results suggest that the inhibitory effect of H-89 on TBKCs was mediated by PKA, resulting in a decrease in Ca\(^{2+}\) spark activity. Thus, the inhibitory effect of H-89 on TBKCs was unlikely to be caused by a direct action on BK channels in UBSM cells (28). PKA-independent inhibitory effect of H-89 on voltage-gated Na\(^{+}\) channels has been reported in rat alveolar type II epithelial cells; however, this should not be a concern for the present study since there are no voltage-gated Na\(^{+}\) channels expressed in guinea pig UBSM cells (25).

PKI 14–22 is a heat-stable peptide inhibitor of PKA, an endogenous polypeptide that interacts specifically with the free catalytic subunit of PKA after dissociation of the holoenzyme (24, 37, 38, 40). PKI 14–22 is a nine-amino acid segment of the polypeptide that resembles the sequence in the “hinge regions” of the PKA regulatory subunits I and II, which bind to the PKA catalytic subunits in vivo and inhibit their enzyme activity (38). Therefore, PKI 14–22 is a highly potent and specific PKA inhibitor (24, 38). The attenuation of TBKCs by PKI 14–22 confirmed that the effect of H-89 was indeed due to the inhibition of the constitutive PKA activity (Fig. 1).

UBSM spontaneous phasic contractions are triggered by spontaneous action potentials (13, 14). Inhibition of BK channels increases both the action potential amplitude and duration and decreases action potential frequency; consequently, this increases the UBSM spontaneous phasic contractions (13, 15, 31). The effects of the constitutive PKA activity on UBSM phasic contractions are consistent with data showing that inhibition of the constitutive PKA activity inhibited the spontaneous TBKCs in UBSM cells (Figs. 1, 2, and 6). The physiological importance of the constitutive activity of PKA in the regulation of UBSM spontaneous contractility was confirmed by the two PKA inhibitors H-89 and KT-5720. We used KT-5720 instead of PKI 14–22 because of its higher membrane permeability. Both H-89 and KT-5720 are ATP-competitive PKA inhibitors; however, they have substantial differences in their chemical structures. The structure of H-89 is much simpler and has more similarity with ATP than KT-5720. KT-5720 increased the spontaneous phasic contractions of freshly isolated UBSM strips, as H-89 did. These data indicate that constitutive PKA activity is functionally important to control the spontaneous contractile activity of UBSM (Fig. 7). The critical role of constitutive PKA activity in maintaining UBSM excitability and contractility is also supported by the observation that a gain of PKA expression counterbalances the increased contractility of mouse UBSM caused by genetic deletion of BK channel pore-forming α-subunit (4, 39).

In conclusion, constitutive PKA activity is fundamentally important for maintaining spontaneous TBKCs and cellular Ca\(^{2+}\) homeostasis in UBSM cells as well as the spontaneous contractility of UBSM, as depicted in Fig. 8.

ACKNOWLEDGMENTS

We thank Drs. John Malyss, Shankar Parajuli, and Kiril L. Hristov and Aaron Provence for critical evaluation of the manuscript.

GRANTS

This study was supported by a grant from the National Institute of Diabetes and Digestive and Kidney Diseases (R01-DK-084284) to G. V. Petkov.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

W.X. and G.V.P. conception and design of research; W.X., N.L., Q.C., V.S.F., and G.V.P. performed experiments; W.X., N.L., Q.C., V.S.F., and G.V.P. analyzed data; W.X., N.L., Q.C., and G.V.P. interpreted results of experiments; W.X., N.L., Q.C., V.S.F., and G.V.P. prepared figures; W.X. and G.V.P. drafted manuscript; W.X., N.L., Q.C., V.S.F., and G.V.P. edited and revised manuscript; W.X., N.L., Q.C., V.S.F., and G.V.P. approved final version of manuscript.

REFERENCES


