Mutation of protein kinase C phosphorylation site S1076 on α-subunits affects BK_{Ca} channel activity in HEK-293 cells

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Submitted 14 October 2008; accepted in final form 3 July 2009

Zhu S, Browning DD, White RE, Fulton D, Barman SA. Mutation of protein kinase C phosphorylation site S1076 on transfected human BK_{Ca} channel α-subunits affects BK_{Ca} channel activity in HEK-293 cells. Am J Physiol Lung Cell Mol Physiol 297: L758–L766, 2009. First published July 10, 2009; doi:10.1152/ajplung.90518.2008.—Large conductance, calcium- and voltage-activated potassium (BK_{Ca}) channels are important modulators of pulmonary vascular smooth muscle membrane potential, and phosphorylation of BK_{Ca} channels by protein kinases regulates pulmonary arterial smooth muscle function. However, little is known about the effect of phosphorylating specific channel subunits on BK_{Ca} channel activity. The present study was done to determine the effect of mutating protein kinase C (PKC) phosphorylation site serine 1076 (S1076) on transfected human BK_{Ca} channel α-subunits in human embryonic kidney (HEK-293) cells, a heterologous expression system devoid of endogenous BK_{Ca} channels. Results showed that mutating S1076 altered the effect of PKC activation on BK_{Ca} channels in HEK-293 cells. Specifically, the phospho-deficient mutation BK_{Ca-α}(S1076A)/β decreased the excitatory effect of PKC activator phosphor bohl myristate acetate (PMA) on BK_{Ca} channels, whereas the phospho-mimetic mutation BK_{Ca-α}(S1076E)/β increased the excitatory effect of PMA on BK_{Ca} channels. In addition, the phospho-null mutation S1076A blocked the activating effect of cGMP-dependent protein kinase G (PKG) on BK_{Ca} channels. Collectively, these results suggest that specific putative PKC phosphorylation site(s) on human BK_{Ca} channel α-subunits influence BK_{Ca} channel activity, which may subsequently alter pulmonary vascular smooth muscle function and tone.

channel subunits

LARGE CONDUCTANCE, CALCIUM- AND VOLTAGE-ACTIVATED POTASSIUM (BK_{Ca}) channels are important regulators of vascular smooth muscle membrane potential. Although multiple classes of K\(^+\) channels are expressed at varying densities in different vascular beds, the BK_{Ca} channel is the predominant K\(^+\) channel species in most arteries (35). BK_{Ca} channels are activated by submicromolar [Ca\(^{2+}\)], and blocked by external charybdotoxin (CTX), iberiotoxin (IBTX), and tetraethylammonium ions (TEA) (35, 38). The biophysical profile of the BK_{Ca} channel is that it is a large conducting channel (100–150 pS in physiological K\(^+\) gradients), which is both calcium- and voltage-dependent for activation (14, 35, 47, 48). Because of their large conductance and high density, these channels influence resting membrane potential and provide an important repolarizing negative feedback mechanism. In pulmonary arterial smooth muscle cells (PASMC), BK_{Ca} channel activation causes K\(^+\) efflux, resulting in membrane hyperpolarization with subsequent closing of calcium channels, leading to a decrease in cytosolic calcium and vascular tone (12, 34, 37).

BK_{Ca} channels are composed of four membrane-spanning subunit complexes aligned in a 1:1 ratio of α-subunits, which form a central pore that is selective for K\(^+\) (1, 28), and tissue-specific β-subunits, which amplify the sensitivity of the channels to activation by physiological changes in Ca\(^{2+}\) (18, 31, 43). Presently, four different subtypes of β-subunits have been identified (37), and in vascular smooth muscle, the β₁-subunit is highly expressed (4, 13, 19, 34, 35). When both subunits are coexpressed, BK_{Ca} currents possess faster activation kinetics and a greater open probability at negative membrane potentials (37). In addition to Ca\(^{2+}\) and voltage, evidence suggests that protein kinases regulate BK_{Ca} channel activity via protein phosphorylation (29, 30). Both cAMP-dependent protein kinase (PKA) and cGMP-dependent protein kinase (PKG) activate BK_{Ca} channels by rendering them more sensitive to Ca\(^{2+}\) (42). Recently, phosphorylation sites for both PKA and PKG have been identified for activation (22, 33), which are fully conserved in all mammalian splice variants (49). PKG opens BK_{Ca} channels through phosphorylation of the channel (2). In HEK-293 cells, BK_{Ca} channel activity is increased by direct phosphorylation of Ser1072 of csl0-α by PKG (22). The effect of protein kinase C (PKC) is less clear as it has been shown that this kinase can either activate, or inhibit, BK_{Ca} channels in different types of vascular smooth muscle (8, 9, 32, 41, 50) as well as activate PKG via phosphorylation through a cGMP-independent pathway in HEK-293 cells (25).

PKC is a key regulatory enzyme involved in the regulation of several cellular functions including vascular smooth muscle growth and contractility (5, 11). PKC consists of a family of serine/threonine kinases with at least 12 members, and numerous PKC isozymes are expressed in vascular smooth muscle, which is dependent on species, vessel type, and age of the vessel (24, 26). In addition, several of these isozymes participate in vasoconstric- tor signaling mechanisms in pulmonary vascular smooth muscle (7, 8). Previous studies document that the effect of PKC on BK_{Ca} channel activity can be due to alteration of the α- and β-subunit channel complex (45, 49), cross-communication with endogenous kinases and phosphatases (23), and activation of specific PKC isozymes (8, 9).

In light of these previous investigations, the present study was done to determine the effect of PKC-dependent phosphorylation on BK_{Ca} channel activity by mutating PKC phosphorylation site S1076 on BK_{Ca} channel α-subunits in transfected human embry- onic kidney (HEK-293) cells, a heterologous expression system that does not possess endogenous BK_{Ca} channels, but can be transfected with specific BK_{Ca} channel subunits to assess BK_{Ca} channel subunit function (19).

MATERIALS AND METHODS

Cell culture. HEK-293 (purchased from American Type Culture Collection) cells were cultured (passage 3 > 10) in MEME media supplemented with 10% vol/vol FBS and grown to ~60% confluence.
at 37°C in a humidified atmosphere containing 5% CO₂ before transfection.

**Site-directed mutagenesis and transfection.** Plasmids of human BKCa channel α-subunits and β₁-subunits were used as the templates. Inspection of the COOH terminus of the human BKCa channel α-subunits revealed the tandem motif HSSQSS₁₀₇₆ SKKSS for phosphorylation by PKC, which is adjacent to a putative phosphorylation site for PKG. Therefore, two mutant BKCa channel α-subunits were created in which S₁₀₇₆ was replaced by alanine (S₁₀₇₆A) or glutamate (S₁₀₇₆E), respectively. S₁₀₇₆A represented the phospho-null mutation, whereas S₁₀₇₆E was the phospho-mimetic mutation. Mutagenesis was obtained by using the QuikChange site-directed mutagenesis kit according to the manufacturer’s instructions (Stratagene). Oligonucleotide primers containing the desired mutation (S to A or E, respectively) were designed. The complementary mutagenic primers used were ACTCGTCGCAGTCCGAAAGCAAGAAGC (for S₁₀₇₆E) and ACTCGTCGCAGTCCGCCAGCAAGAAGC (for S₁₀₇₆A). Wild-type and mutant BKCa subunits were then sequence verified by the Sanger method.

**RESULTS**

**Mutations of PKC phosphorylation site (S₁₀₇₆) alter the effect of PKC on BKCa channels.** Inspection of the human BKCa channel α-subunits and β₁-subunits for putative phosphorylation sites using the NetPhosK online server (10) revealed a consensus PKC phosphorylation site in the COOH terminus (HSSQSS₁₀₇₆ SKKSS), which is adjacent to a putative site for PKG. To study the significance of this specific site on BKCa channel regulation by PKC and PKG, two mutant BKCa channels were created in which S₁₀₇₆ was replaced by alanine (S₁₀₇₆A) or glutamate (S₁₀₇₆E), respectively. S₁₀₇₆A represented the phospho-null mutation, whereas S₁₀₇₆E was the phospho-mimetic mutation.

The effect of PKC on BKCa channels in wild-type BKCa-α/β₁, BKCa-α(S₁₀₇₆A)/β₁, and BKCa-α(S₁₀₇₆E)/β₁ subunit-transfected HEK-293 cells is illustrated in Figs. 1–3. In cells transfected with wild-type BKCa channels, 100 nM PMA activated BKCa channels by 42-fold (NPₒ: control 0.0008 ± 0.0001; PMA 0.0338 ± 0.0108; n = 3; +40 mV; P < 0.05) compared with control (Fig. 1). However, as shown in Fig. 2, in HEK-293 cells transfected with phospho-deficient (phospho-null) BKCa channels [BKCa-α(S₁₀₇₆A)/β₁], 100 nM PMA increased BKCa channel activity only ~2-fold compared with control (NPₒ: control 0.0004 ± 0.0001; PMA 0.0048 ± 0.0008; n = 3; +40 mV; P < 0.05), demonstrating that the phospho-deficient mutation significantly attenuated the activating effect of PKC on BKCa channels compared with the effect of PMA on the wild-type BKCa channels (Fig. 1). In contrast, the phospho-mimetic mutation BKCa-α(S₁₀₇₆E)/β₁ increased the excitatory effect of PMA on BKCa channels by ~50-fold (NPₒ: control 0.0174 ± 0.0012; PMA 0.0867 ± 0.0389; n = 3; +40 mV; P < 0.05) (Fig. 3), which is similar to the magnitude increase of the heightened response observed with PMA (42-fold) on the wild-type BKCa channels (Fig. 1). Furthermore, the PKC inhibitor chelethyrine (100 nM; pretreatment for 30 min) blocked the excitatory response to PMA on BKCa channels in both the wild-type (NPₒ: chelethyrine alone 0.0185 ± 0.0069; chelethyrine + PMA 0.0282 ± 0.0143) and phospho-mimetic BKCa-α(S₁₀₇₆E)/β₁-transfected HEK-293 cells (NPₒ: chelethyrine 0.0227 ± 0.0148; chelethyrine + PMA 0.0176 ± 0.0024) (Fig. 4).

In experiments done with HEK-293 cells transfected with only α-subunits (hslo), there was no discernable effect of PMA (data not shown), which indicates that β₁-subunits are neces-
effect of PKC phosphorylation on BKCa channel activity

Fig. 1. The effect of protein kinase C (PKC) activation on large conductance, calcium- and voltage-activated potassium (BKCa) channels in wild-type BKCa-α/β1-transfected HEK-293 cells. In cells transfected with human wild-type BKCa channel subunits, 100 nM phorbol myristate acetate (PMA) activated BKCa channels by 42-fold (n = 3; P < 0.05) compared with control conditions. A: continuous recording from the same cell-attached patch (+40 mV) before and 10 min after application of 100 nM PMA in wild-type BKCa-α/β1-transfected HEK-293 cells. Channel openings are upward deflections from baseline (closed state; dashed line). B: activity plot of channel open probability (NPo) before and 10 min after application of 100 nM PMA. Total recording time under each condition was 10–11 s, as indicated on the time axis. The break in the time axis represents drug incubation periods. Horizontal lines above the histogram indicate period of drug exposure.

Mutations of PKC phosphorylation site (S1076) alter the effect of PKC on BKCa channels. In HEK-293 cells that were transfected with GFP alone, no BKCa channel activity was observed under control conditions or after the application of 100 nM PMA (data not shown), which is consistent with the premise that there is no effect of PKC activation on BKCa channel activity in HEK-293 cells that do not exhibit endogenous BKCa channel expression. However, PMA does appear to induce a small but significant transient increase in intracellular Ca2+ in HEK-293 cells (Fig. 5) suggesting that the PMA-induced excitatory effect on BKCa channel activity may involve minor fluctuations in cytoplasmic Ca2+. Collectively, these results indicate that the putative PKC phosphorylation site S1076 on the COOH terminus of human BKCa channel α-subunits influences the regulation of BKCa channel function.

Mutations of PKC phosphorylation site (S1076) alter the effect of PKG on BKCa channels. Cell-free patch recordings were done on both wild-type BKCa-α/β1 and BKCa-α(S1076A)/β1-transfected HEK-293 cells. As illustrated in Fig. 6, lowering calcium concentration from 100 to 0.1 μM decreased BKCa channel activity significantly, and subsequent application of 400 units of PKG with 15 μM cGMP and 5 mM ATP activated BKCa channels in the wild-type (NPo: control 0.0021 ± 0.0009; PMA + cGMP + ATP 0.0683 ± 0.0226; n = 3; +40 mV; P < 0.05), but not in the phospho-null mutation configuration (NPo: control 0.0057 ± 0.008; PMA + cGMP + ATP ~0; n = 3; +40 mV). Furthermore, as shown in Fig. 7, exposure of the BKCa-α(S1076E)/β1-transfected HEK-293 cells to CPT-cGMP (cell-permeable cGMP analog) also increased BKCa channel activity (NPo: control 0.0280 ± 0.0040; CPT-cGMP 0.3050 ± 0.0237; n = 3; +40 mV; P < 0.05).

Figure 8A shows that treatment with the PKG inhibitor KT5823 attenuated the effect of PMA on BKCa channel open probability (NPo: KT5823 alone 0.0236 ± 0.0082, KT5823+PMA 0.1273 ± 0.0123; 5-fold increase) compared with PMA alone in the wild-type cells (42-fold increase), whereas a similar phenomenon was observed in the phospho-mimetic state as shown in Fig. 8B (NPo: KT5823 alone 0.0476 ± 0.0288, KT5823+PMA 0.1947 ± 0.0460) (4-fold increase vs. 50-fold increase with PMA alone). Calcium (100 μM) was able to restore channel activity in the BKCa-α(S1076A)/β1-transfected cells (Fig. 6), demonstrating that mutating S1076 to the phos-
pho-null state did not effect the viability of the BKCa channels in this cell system.

BKCa channels remain active in phospho-mimetic mutation (S1076E) under low [Ca2+] conditions. In cell-free (inside-out) patches, BKCa channels normally stay in an inactive state when exposed to a low-calcium concentration (0.1 μM, Fig. 6). However, as seen in Fig. 9, cell-free patches from BKCa-α(S1076E)/β1-transfected HEK-293 cells remained active (NPo: 0.0857 ± 0.0305) even after lowering calcium concentration from 100 to 0.1 μM, indicating an increased sensitivity to calcium. To verify that recorded channel activity was primarily due to BKCa channels, 1 mM TEA (a specific BKCa channel inhibitor at this concentration) was applied, which completely blocked BKCa channel activity (NPo: ~0; n = 3; +40 mV; P < 0.05).

DISCUSSION

The results of this study showed that mutating the specific PKC phosphorylation site S1076 altered the effect of PKC activation on BKCa channels in HEK-293 cells, an expression system that does not possess endogenous BKCa channels. Specifically, it was found that: 1) the phospho-deficient mutation BKCa-α(S1076A)/β1 attenuated the activating effect of the PKC agonist PMA on BKCa channels, 2) the phospho-mimetic mutation BKCa-α(S1076E)/β1 mimicked the excitatory effect of PMA on BKCa channel wild-type subunits, 3) the phospho-null mutation (S1076A) blocked the stimulatory effect of PKG on BKCa channels, 4) the PKC inhibitor chelethyrine blocked the excitatory response to PMA on BKCa channels in both the wild-type and phospho-mimetic HEK-293 cells, 5) exposure of the BKCa-α(S1076E)/β1-transfected HEK-293 cells to cGMP (cell-permeable cGMP analog) increased BKCa channel activity, and 6) the PKG inhibitor KT5823 attenuated the effect of PMA on BKCa channel P0 compared with PMA alone in both the wild-type and phospho-mimetic states. Collectively, these results suggest that phosphorylating site S1076 on the COOH terminus of human BKCa channel α-subunits influences the effect of protein kinases on BKCa channel function.

BKCa channels are composed of four membrane-spanning subunit complexes consisting of α-subunits and tissue-specific β-subunits arranged in a 1:1 stoichiometric ratio (1, 28, 43). In vascular smooth muscle, the β1-subunit is highly expressed, which amplifies the sensitivity of BKCa channels to physiological changes in Ca2+ (18, 31). Specifically, when both subunits...
are coexpressed, BKCa currents possess faster activation kinetics and a greater open probability at negative membrane potentials (37). The mechanisms for this apparent enhanced Ca²⁺ sensitivity due to β₁-subunit coupling are not well understood, although most studies agree that an alteration of calcium binding to the BKCa channel is not involved (6, 18). Rather, it is thought that these subunits shift the equilibrium for voltage-sensor activation to more negative potentials, which decrease the work necessary for Ca²⁺ binding to open the channel (17). Amberg and Santana (4) found that BKCa channels from spontaneous hypertensive rats were less sensitive to physiological changes in Ca²⁺ due to decreased expression of β₁-subunits, suggesting that the molecular composition of BKCa channels may be important in altered vascular function in pathophysiological conditions. Deletion of β₁-subunits in mice results in membrane depolarization and the complete lack of spontaneous transient outward currents in isolated blood vessels (40).

Our results showed that PKC activation increased BKCa channel openings in transiently transfected wild-type HEK-293 cells, which supports evidence suggesting that protein kinases regulate BKCa channel activity. PKC can either activate or inhibit BKCa channels (8, 9, 41, 44, 50), and both PKA and PKG activate these channels by increasing the sensitivity of the channel to Ca²⁺ (42). Furthermore, Kim and Park (27) ob-

![Fig. 5. Effect of PMA on cytoplasmic Ca²⁺ in HEK-293 cells. HEK-293 cells were transfected with wild-type (wt) aequorin and challenged with 100 nM PMA or 1 μM ionomycin (positive control). PMA does appear to induce a small but significant transient increase in intracellular Ca²⁺ (n = 6).](http://ajplung.physiology.org/)

![Fig. 6. The effect of PKG on BKCa channels in wild-type BKCa-α/β₁-transfected HEK-293 cells. A: lowering calcium concentration from 100 to 0.1 μM decreased BKCa channel activity significantly, and application of 400 units of PKG with 15 μM cGMP and 5 mM ATP activated BKCa channels in wild-type channels (n = 3; P < 0.05). All panels are consecutive recordings from the same inside-out patch (+40 mV) in wild-type BKCa-α/β₁-transfected HEK-293 cells, respectively. Channel activity shown in 100 μM (left) or 0.1 μM [Ca²⁺] (middle). Channel activity shown 30 min after addition of 400 U/ml PKG with 15 μM cGMP and 5 μM ATP (right). Channel openings are upward deflections from baseline (closed state; dashed line). B: phospho-null mutation of PKC phosphorylation site (S1076A) blocks the activation effect of PKG on BKCa channels. Lowering calcium concentration from 100 to 0.1 μM decreased BKCa channel activity significantly, and additional application of 400 units of PKG with 15 μM cGMP and 5 mM ATP does not activate BKCa channels in BKCa-α(S1076A)/β₁-transfected cells (n = 3). Calcium (100 μM) was able to restore channel activity, which verified the continued viability of BKCa channels in these cells (right). All panels are consecutive recordings from the same inside-out patch (+40 mV) in BKCa-α(S1076A)/β₁-transfected HEK-293 cells, respectively. BKCa channel activity is shown in 100 μM (left) or 0.1 μM [Ca²⁺] (left inner). BKCa channel activity shown 30 min after addition of 400 U/ml PKG with 15 μM cGMP and 5 μM ATP (right inner). Channel openings are upward deflections from baseline (closed state; dashed line).](http://ajplung.physiology.org/)
served that PKC activation by PDBu, a cell-permeable PKC activator, increased BK<sub>Ca</sub> channel activity in COS7 cells by phosphorylating the cytosolic COOH-terminal domain of the α-subunit. Specific to this study, in HEK-293 cells transfected with phospho-deficient (phospho-null) BK<sub>Ca</sub> channels [BK<sub>Ca</sub>-α(S1076A)/β<sub>1</sub>], PMA increased BK<sub>Ca</sub> channel activity ~12-fold compared with control (wild-type) conditions, demonstrating that the phospho-null mutation significantly attenuated the activating effect of PMA on BK<sub>Ca</sub> channels compared with the response on the wild-type BK<sub>Ca</sub> channels. In contrast, the phospho-mimetic mutation BK<sub>Ca</sub>-α(S1076E)/β<sub>1</sub> increased the excitatory effect of PMA on BK<sub>Ca</sub> channels by ~50-fold, which is similar in magnitude to the response observed with PMA (42-fold) on the wild-type BK<sub>Ca</sub> channels. The reasons why PMA still increased BK<sub>Ca</sub> channel activity in the phospho-null HEK-293 cells with respect to the wild-type (control) conditions are not clear. However, based on consensu target sequence information for PKC, analysis identified three putative PKC phosphorylation sites clustered at the COOH terminus of the α-subunit that are conserved among species with no conserved phosphorylation sites present on the β<sub>1</sub>-subunit. Since only one of the PKC phosphorylation sites (S1076) on the COOH terminus of the BK<sub>Ca</sub>-α-subunit was mutated, PMA may still have had an effect on the other

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Fig. 7. PKG increases BK<sub>Ca</sub> channel activity in phospho-mimetic HEK-293 cells. Exposure of BK<sub>Ca</sub>-α(S1076E)/β<sub>1</sub>-transfected HEK-293 cells to CPT-cGMP (cell-permeable cGMP analog) increased BK<sub>Ca</sub> channel activity (n = 3; +40 mV; *P < 0.05). Continuous recording from the same cell-attached patch (+40 mV) before and 10 min after application of 100 μM CPT-cGMP in BK<sub>Ca</sub>-α(S1076E)/β<sub>1</sub>-transfected HEK-293 cells. Channel openings are upward deflections from baseline (closed state; dashed line). Activity plot of channel open probability before and 10 min after application of 100 μM CPT-cGMP. Total recording time under each condition was 10–11 s. *P < 0.05.

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Fig. 8. Effect of PKG inhibition on BK<sub>Ca</sub> channels in wild-type and phospho-mimetic BK<sub>Ca</sub>-α/β<sub>1</sub>-transfected HEK-293 cells. A: the PKG inhibitor KT5823 modulated the effect of PMA on BK<sub>Ca</sub> channel open probability (~5-fold increase), which is significantly less compared with PMA alone in the wild-type cells (42-fold increase, Fig. 1). B: similar phenomenon was observed in the phospho-mimetic state with KT5823 (4-fold increase vs. 50-fold increase with PMA alone, Fig. 3). Channel activity shown 30 min after addition of 300 nM KT5823 and 100 nM PMA (n = 3). Channel openings are upward deflections from baseline (closed state; dashed line). Activity plot of channel open probability before (KT5823 alone) and 30 min after application of 100 nM PMA (n = 3). Total recording time under each condition was 10–11 s.
Fig. 9. BK<sub>Ca</sub> channels remain active in phospho-mimetic mutation (S1076E) under low [Ca<sup>2+</sup>] conditions. BK<sub>Ca</sub>-α(1)(S1076E)/β₁-transfected HEK-293 cells remained active even after lowering calcium concentration from 100 (left) to 0.1 μM (middle). Tetraethylammonium (TEA; a specific BK<sub>Ca</sub> channel inhibitor at this concentration) (1 mM) completely blocked BK<sub>Ca</sub> channel activity (n = 3; P < 0.05) (right). All panels are consecutive recordings from the same inside-out patch (+40 mV), and channel openings are upward deflections from baseline (closed state; dashed line).

EFFECT OF PKC PHOSPHORYLATION ON BK<sub>Ca</sub> CHANNEL ACTIVITY

The current study did not address which specific PKC isozymes are involved in BK<sub>Ca</sub> channel subunit function in the HEK-293 cells. PMA, a phorbol ester, is a relatively nonspecific PKC activator that exerts its effect by substituting for diacylglycerol (15). Kim and Park (27) recently found that four specific PKC isozymes (α, δ, γ, θ) phosphorylated the COOH terminus of Slo1 and purified PKCδ-activated BK<sub>Ca</sub> channels in excised patches of transfected HEK-293 cells. Furthermore, Hou and colleagues (25) suggested that since PKCα is widely expressed in HEK-293 cells, this particular isozyme might be involved in phosphorylation of PKG.

Studies show that the pulmonary vasculature expresses both α and β₁ BK<sub>Ca</sub> channel subunits (37). Since BK<sub>Ca</sub> channels are important regulators of vascular tone and systemic blood pressure (17), and that changes in β₁-subunit expression may be involved in vascular dysfunction during hypertension (3, 4), it is reasonable to speculate that these ion channels are important determinants of pulmonary arterial pressure and that alteration in expression and/or function of these subunits could be a primary signaling mechanism in the development of pulmonary hypertension.

In summary, this study showed that mutations of specific PKC phosphorylation site S1076 altered the effect of PKC on BK<sub>Ca</sub> channels in HEK-293 cells as the phospho-deficient mutation BK<sub>Ca</sub>-α(S1076A)/β₁ attenuated the activating effect of PMA on BK<sub>Ca</sub> channels, whereas the phospho-mimetic mutation BK<sub>Ca</sub>-α(S1076E)/β₁ increased the excitatory effect of PMA on BK<sub>Ca</sub> channels. In addition, the phospho-null mutation of PKC phosphorylation site (S1076A) blocked the activation effect of PMA on BK<sub>Ca</sub> channels. Collectively, these results suggest that either phosphorylating or dephosphorylating putative PKC phosphorylation sites on the COOH terminus of human BK<sub>Ca</sub> channel α-subunits influences the regulation of protein kinases on BK<sub>Ca</sub> channel activity, which may subsequently alter pulmonary vascular smooth muscle function and tone under pathophysiological conditions.

ACKNOWLEDGMENTS

We gratefully acknowledge the technical assistance of Louise Meadows.
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GRANTS

This work was supported by National Heart, Lung, and Blood Institute Grants HL-68026 (S. A. Barman) and HL-64779 (R. E. White) and American Heart Association Southeast Affiliate Grant-in-Aid Award 5550149B (S. A. Barman).

REFERENCES

6. Bao L, Cox DH. Gating and ionic currents reveal how the BK<sub>Ca</sub> channel's Ca<sup>2+</sup> sensitivity is enhanced by its β1 subunit. J Gen Physiol 126: 393–412, 2005.
33. Hagem BM, Bayguinov O, Sanders KM. β-Subunits are required for regulation of coupling between Ca<sup>2+</sup> transients and Ca<sup>2+</sup>-activated K<sup>+</sup> (BK) channels by protein kinase C. Am J Physiol Cell Physiol 285: C1270–C1280, 2003.