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Role of phosphodiesterases in modulation of BK_{Ca} channels in hypertensive pulmonary arterial smooth muscle

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Abstract

BK_{Ca} channels regulate pulmonary arterial pressure, and protein kinase C (PKC) inhibits BK_{Ca} channels, but little is known about PKC-mediated modulation of BK_{Ca} channel activity in pulmonary arterial smooth muscle. Studies were done to determine mechanisms of PKC modulation of BK_{Ca} channel activity in pulmonary arterial smooth muscle cells (PASMC) of the Fawn-hooded rat (FHR), an animal model of pulmonary hypertension. Forskolin opened BK_{Ca} channels in FHR PASMC, which was blocked by PKC activation, and reversed by the phosphodiesterase (PDE) inhibitors IBMX, milrinone, and zaprinast. PDE inhibition also blocked the vasoconstrictor response to PKC activation in FHR pulmonary arteries. These results indicate that PKC inhibits cAMP-induced activation of BK_{Ca} channels and causes pulmonary vasoconstriction in hypertensive pulmonary arterial smooth muscle via PDE, which further suggests PDE inhibitors for treatment of pulmonary hypertension.

Keywords

phosphodiesterase; BK_{Ca} channels; PKC; cAMP; pulmonary

INTRODUCTION

Signal transduction pathways defining the role of cyclic nucleotides in the regulation of vascular tone is currently an area of great interest. Agents that relax vascular smooth muscle via activation of adenylate cyclase, such as forskolin, are thought to elicit their biological effects by increasing the intracellular concentration of cAMP [Haynes et al, 1991, Haynes et al, 1992, Sakai and Voelkel, 1988]. Although the exact mechanism by which cAMP causes vascular relaxation is not known, it is believed that activation of PKA is involved [Conti and Adelstein, 1981, Haynes et al, 1992, Hei et al, 1991]. In addition to cAMP-mediated vascular relaxation, guanosine-3',5'-cyclic monophosphate (cGMP) has been reported to elicit vasodilatation by activation of cGMP-dependent PKG [Carrier et al, 1997, Lincoln and Cornwell, 1993]. In the pulmonary circulation, cGMP and cAMP have been implicated as mediators of smooth muscle vasodilatation [Haynes et al, 1991, Francis et al, 1988, Gao et al, 1999, Lincoln et al, 1990], and in ovine pulmonary vascular smooth muscle, it has

recently been reported that pulmonary arteries are more sensitive to vasorelaxation induced by cGMP, and that activation of PKG is involved in both cGMP- and cAMP-induced vasorelaxation [Dhanakoti et al, 2000].

Recent evidence suggests that the vasodilatory effects of cAMP-producing agents may involve “cross-activation” of PKG by cAMP [Toyoshima et al, 1988, White et al, 1995]. Lincoln and colleagues [Lincoln et al, 1990] originally showed that cross-activation of PKG by cAMP was important in cAMP-elevating agent vasodilatation, and earlier studies have shown that agents such as forskolin can activate PKG by increasing cAMP levels in both vascular and nonvascular smooth muscle [Hei et al, 1991, Francis et al, 1988, Lincoln et al, 1990]. Barman et al [Barman et al, 2003] showed that the cAMP-dependent vasodilators forskolin (activator of adenylate cyclase) and CTP-cAMP (membrane-permeable derivative of cAMP) activate BK_{Ca} channels in hypertensive pulmonary arterial smooth muscle cells (PASMC) of the Fawn-Hooded rat (FHR) via PKG-dependent and PKA-independent signaling pathways, which suggests cross-activation between cyclic nucleotide-dependent protein kinases in pulmonary arterial smooth muscle. More recently it was shown that PKC activation inhibits forskolin-induced BK_{Ca} channel activity in FHR PASMC [Barman et al, 2004].

Vasodilators increase the intracellular concentration of cAMP or cGMP via activation of adenylyl cyclase (AC) or guanylate cyclase (GC), respectively [Beavo, 1995]. Phosphodiesterases (PDEs) represent a superfamily of 11 distinct isozymes that inactivate cAMP and/or cGMP, with tissue-specific distribution [Ahn et al, 1991, Rabe et al, 1994]. PDE I, III, IV and V have been identified in the pulmonary vasculature [Rabe et al, 1994], with PDE III and PDE V being most abundant in pulmonary arteries [Maclean et al, 1997, Matot and Gozal, 2004].

In light of these previous investigations, the present study was done to determine the role of phosphodiesterase (PDE) signaling in pulmonary arterial smooth muscle. Specifically, the effect of PDE inhibition on PKC-induced pulmonary arterial vasoconstriction and inhibition of forskolin-induced BK_{Ca} channel activity was investigated in hypertensive pulmonary arterial smooth muscle of the Fawn-Hooded rat (FHR), an animal model of pulmonary hypertension. Our previous studies showed that PKC activation inhibited the effect of the cAMP-elevating vasodilator forskolin on BK_{Ca} channel activity [Barman et al, 2004]. Since PDE can hydrolyze cAMP and/or cGMP, we hypothesized that PKC may act as a PDE or activate PDEs to inactivate cAMP and/or cGMP to inhibit cAMP-induced BK_{Ca} channel activity in FHR PASMC.

MATERIALS AND METHODS

Animals

All procedures and protocols were approved by the Animal Care and Use Committee at the Medical College of Georgia. FHRs were a gift from Dr. I. McMurtry (Center for Lung Biology, University of South Alabama, Mobile, AL), and a breeding colony was established. Animals were maintained on standard rat chow, allowed free access to food and water, and

exposed to a 12:12-h light-dark cycle. Male FHRs at 12–16 wk of age were used for this study.

Isolation of PASMC

After the rats were anesthetized with pentobarbital sodium (50 mg/kg ip), a midline incision was made, and the lungs were immediately excised and placed into ice-cold dissociation buffer consisting of (in mM): 110 NaCl, 5 KCl, 0.16 CaCl₂, 2 MgCl₂, 10 HEPES, 0.5 NaH₂PO₄, 10 NaHCO₃, 0.5 KH₂PO₄, 10 glucose, 0.49 EDTA, and 10 taurine. Pulmonary arterial vessels (0.5–1.0mm diameter) were dissected from the lung lobes under a stereomicroscope, endothelium was removed, and the adventitia was carefully teased away. The vascular tissue was then dissociated enzymatically at 35°C for 1h in an incubation solution of dissociation buffer containing the following: papain (6.7 mg/5 ml), cysteine (3.5 mg/5 ml), and BSA (5.0 mg/ml). After incubation, 5.0 ml of dissociation buffer containing 2.0 mg/ml BSA was added to the cell solution, and the tissue was then triturated gently, which allowed individual smooth muscle cells to fall away from the larger pieces of undigested tissue with minimal damage to the cells. These cells exhibited morphology characteristics of vascular smooth muscle cells. The solution was removed and centrifuged at 1,200 rpm for 10 min, and the pellet was then resuspended in dissociation buffer. For the patch-clamp experiments, several drops of cell suspension were placed in a microscope chamber containing recording solution, and all experiments were performed within 1–2 h after cell dissociation.

Single-channel experiments

Single potassium channels were measured in cell-attached patches by filling the patch pipette (2–5 MΩ) with normal Ringer solution and making a gigaohm seal on an intact cell. The solution in the recording chamber contained 140mM KCl, 10mM MgCl₂, 0.1mM CaCl₂, 10mM HEPES, and 30mM glucose (pH 7.4). In experiments measuring potassium channel activity in cell-free inside-out patches, the solution facing the cytoplasmic surface of the membrane had the following composition: 115mM KCH₃SO₃, 26mM KCl, 2mM MgCl₂, 1mM BAPTA, 0.47mM CaCl₂ (pCa 7), 5.0mM Mg-ATP, 0.1mM GTP, and 20mM HEPES (pH 7.4). The solution facing the external surface was the standard recording solution. Voltage-clamp and voltage pulse generation was controlled with an Axopatch 200A patch-clamp amplifier (Axon Instruments), and data was analyzed with pCLAMP 6.0.3 (Axon Instruments), which is a comprehensive software package for acquisition and analysis of both whole cell and single-channel currents. Voltage-activated currents were filtered at 2 kHz and digitized at 10 kHz, and capacitive and leakage currents were subtracted digitally. All drugs were diluted into fresh bath solution (1ml) and perfused into a 2ml recording chamber (Warner Instruments). Channel activity was quantified by calculating the single channel open probability (*N*P_o) over the duration of the entire data file, as described previously [Carrier et al, 1997, White et al, 1995, Dimitropoulou et al, 2002, White et al, 2000].

Isolated vessel preparation

Fawn-Hooded rats (FHR) were anesthetized with Na-pentobarbital (20mg/kg ip), intubated, and injected with heparin (100U in the left ventricle). A midline incision was made and the lung lobes were excised. Pulmonary arterial segments were dissected from the isolated lobe, and pulmonary arterial vessels (0.5–1mm diameter) were isolated microscopically, cleaned of perivascular tissue, and cut into 1–2-mm rings. The endothelial cell layer was removed as previously described with fine forceps [Carrier and White, 1985]. To ensure that the vessels were denuded, the inability of acetylcholine to produce endothelium-dependent relaxation was assessed. Vessels that did not relax to acetylcholine after initially being constricted with phenylephrine was pharmacological evidence that the endothelium was removed. Isometric tension was measured and recorded with a Radnoti digital force transducer organ bath system (Model 159920) in ring preparations as previously described [Carrier et al, 1997]. The tissue was bathed in a Krebs solution of the following composition: 120mM NaCl, 4.8mM KCl, 18mM NaHCO₃, 1.2mM MgCl₂, 2.5mM CaCl₂, and 11mM glucose, and the solution was oxygenated with 95% O₂/5% CO₂ and maintained at 37°C. The pulmonary vessel preparations were equilibrated 90 min under control conditions, and the resting tension level where the maximum contractile response to phenylephrine was obtained was considered the optimal resting tension. After the initial equilibration period, the vessels were exposed to a maximally effective concentration of 10μM phenylephrine. After a stable tension plateau developed, the phenylephrine was removed by several washes and a period of 30 min was allowed for re-equilibration back to baseline tension levels. This procedure was repeated until the steady-state tension level remained constant [Carrier et al, 1997], which allowed for stabilization of the vascular smooth muscle. Isometric contractions were normalized by expressing force developed per cross-sectional area (g/mm²), which took into account the variation in size of the vessels.

Measurement of PDE activity

PDE activity was measured by a BIOMOL GREEN™ QuantiZyme™ Assay System (AK-800). Briefly, pulmonary arteries were obtained and prepared as described in *Isolation of PASMC*. Tissues were exposed to different treatments (see below). After incubation, tissues were rapidly frozen in liquid nitrogen, pooled and pulverized (Fisher Scientific). Subsequently, homogenization buffer containing protease inhibitors (in mM) 50 Tris-HCl (pH 7.7), 0.1 EDTA, 1 EGTA, 250 sucrose, 0.1% 2-mercaptoethanol, 10% glycerol, 1 phenylmethylsulfonyl fluoride, 1 pepstatin A, 2 leupeptin, and 0.1% aprotinin, were added. All reagents were purchased as a complete PDE assay kit from BIOMOL® International, LP, and cAMP or cGMP was added as the substrates. When hydrolyzed by PDE, 5′-nucleotides were released, and additional application of 5′-nucleotidase cleaved 5′-nucleotides into nucleoside and phosphate. The phosphate was quantified using BIOMOL GREEN™ reagent in a modified Malachite Green assay (Microplate reader, 620nm). Protein concentrations of tissue samples were determined by Bio-Rad DC protein assay.

Experimental Protocols

FHR isolated pulmonary arterial vessel treatments using PMA (100nM) were done with: 1) PMA alone (n = 4), 2) pretreatment with isobutylmethylxanthine (IBMX, 100μM) for 30

min before PMA ($n = 4$), 3) pretreatment with the specific PDE III inhibitor milrinone ($10\mu\text{M}$) for 30 min before PMA ($n = 4$), and 4) pretreatment with the specific PDE V inhibitor zaprinast ($10\mu\text{M}$) for 30 min before PMA ($n = 4$). For the electrophysiological experiments, FHR PASMCM were treated with either: 1) forskolin alone ($10\mu\text{M}$) for 30 min ($n = 4$), or 2) with thymeleatoxin (100nM) in the presence of IBMX, milrinone, or zaprinast for 30 min before the addition of forskolin for 30 min ($n = 4$ for each group). For measurement of PDE III and PDE V activity, FHR pulmonary arterial vessel tissue was treated with PMA for 30 min before assaying for either PDE III or PDE V.

Drugs

Thymeleatoxin, and milrinone were purchased from Calbiochem. Phorbol 12-myristate13-acetate (PMA) was purchased from Alexis. All other agents were purchased from Sigma Chemical.

Statistical analysis

All data were expressed as means $\pm$ SE. Statistical significance between two groups was evaluated by Student's *t*-test for paired data. Comparison among multiple groups was made by using one-way ANOVA for multiple comparisons. A *P* value < 0.05 indicated a significant statistical difference.

RESULTS

PDE inhibitors reverse the inhibitory effect of PKC activation on BK_{Ca} channels

The effect of the PKC isozyme activator thymeleatoxin (THX) on forskolin-induced BK_{Ca} channel open probability (NPo) is shown in figure 1. Forskolin (FSK) initially increased BK_{Ca} channel activity, which was subsequently blocked by the addition of 100nM THX after 15–30 minutes of treatment. As seen in figure 2 (left panel), FHR PASMCMs were pre-treated with $100\mu\text{M}$ IBMX (non-specific PDE inhibitor) and 100nM thymeleatoxin for 30 minutes, and cell-attached patch clamp recordings were performed, which resulted in no apparent BK_{Ca} channel activity ($\text{NPo}=0 \pm 0$). The subsequent application of $10\mu\text{M}$ FSK in the presence of both IBMX and THX increased BK_{Ca} channel activity significantly ($\text{NPo}=0.1061 \pm 0.0306$, $n=3$, $p<0.05$) indicating that non-specific inhibition of PDEs reverses the antagonistic effect of PKC on BK_{Ca} channel activity in hypertensive pulmonary arterial smooth muscle.

The effect of a specific PDE III inhibitor milrinone on forskolin-induced BK_{Ca} channel activity is shown in figure 3. FHR PASMCMs were pre-treated with $10\mu\text{M}$ milrinone and 100nM THX for 30 min ($\text{NPo}=0.0008\pm0.0007$), and the cumulative addition of $10\mu\text{M}$ FSK stimulated BK_{Ca} channels to an NPo of 0.2853 ± 0.1794 , ($n=3$, $p<0.05$), indicating that specific inhibition of PDE III reverses the effect of PKC on BK_{Ca} channel activity.

The effect of a specific PDE V inhibitor zaprinast on forskolin-induced BK_{Ca} channel activity is shown in figure 4, whereby pre-treatment with $10\mu\text{M}$ zaprinast and 100nM THX for 30 minutes had little effect on channel open probability ($\text{NPo}=0.0006 \pm 0.0006$). Subsequent addition of $10\mu\text{M}$ FSK increased NPo to 0.0422 ± 0.0073 ($n=3$, $p<0.05$),

indicating that specific inhibition of PDE V reverses the negating effect of PKC on BK_{Ca} channel activity. Finally, to demonstrate FHR PASMC viability in the presence of PKC activation, figure 5 shows that 100nM THX (pretreatment for 30 minutes) inhibited the effect of FSK on BK_{Ca} channel NPo. Subsequent excision of the patch into an inside-out configuration and raising cytoplasmic Ca²⁺ concentration produced an immediate increase in BK_{Ca} channel NPo in the presence of both THX and FSK, indicating the continued BK_{Ca} channel viability in these cells.

PDE inhibitors decrease the contractile response to PKC activation in isolated FHR pulmonary arterial vessels—

The effect of PDE inhibition on PMA-induced vasoconstriction (measured as % of baseline tension) in isolated FHR pulmonary arterial vessels is shown in figures 6–8. PMA caused vasoconstriction in FHR pulmonary arteries, increasing the contractile response by approximately 150–175% over control tension levels. Pre-treatment with the non-specific PDE inhibitor IBMX (100μM) for 30 minutes significantly attenuated the vasoconstrictor response in the hypertensive vessels to approximately 15% of the maximal contractile response to PMA (figure 6). In addition, pre-treatment with the PDE III inhibitor milrinone (10μM) for 30 minutes resulted in an approximate 20% maximal contractile response to PMA (figure 7), and the PDE V inhibitor zaprinast (10μM; 30 minutes) decreased the vasoconstrictor response to PMA to approximately 45% (figure 8). All three PDE inhibitors alone had no effect on baseline vessel tension.

Activation of PKC increases PDE activity—Biochemical assay of PDE III and PDE V activity in FHR pulmonary arterial smooth muscle is shown in figure 9, which shows that treatment with 100nM PMA for 30 minutes significantly increased PDE III ($98.75 \pm 22.98\%$, $n=4$, $p<0.05$) and PDE V ($95.25 \pm 28.60\%$, $n=4$, $p<0.05$) activity above baseline levels.

DISCUSSION

The results of the present study show that the non-selective phosphodiesterase (PDE) inhibitor isobutylmethylxanthine (IBMX), the specific PDE III inhibitor milrinone, and the specific PDE V inhibitor zaprinast reverse the inhibitory effect of PKC activation by thymeleatoxin on BK_{Ca} channels in FHR PASMC, and inhibit the vasoconstrictor response to PMA in FHR isolated pulmonary arteries. In addition, PMA significantly increases both PDE III and PDE V activity in FHR PASMC. To our knowledge, this is the first study to suggest a causal relationship between PKC and phosphodiesterase signaling in hypertensive pulmonary arterial smooth muscle.

Evidence suggests that PKC inhibits BK_{Ca} channels in pulmonary and other types of arterial smooth muscle. Recently, Barman and colleagues [Barman et al, 2004] reported that PKC activation inhibits forskolin-induced BK_{Ca} channel activity in FHR PASMC. Minami et al. [Minami et al, 1993] observed that PKC blocks activation of BK_{Ca} channels in coronary artery smooth muscle, and Schubert and colleagues [Schubert et al, 1999] reported that PKC reduces BK_{Ca} current in rat tail artery smooth muscle cells. Barman [Barman, 1999] also showed that BK_{Ca} channels modulate the canine pulmonary vasoconstrictor response to

PKC activation, and Taguchi et al. [Taguchi et al, 2000] observed that PKC inhibits BK_{Ca} channels in cultured rat mesenteric artery smooth muscle cells.

The results of this study suggest involvement of PDE III and PDE V in PKC signaling in hypertensive pulmonary arterial smooth muscle. Earlier studies report that the primary PDE isoenzymes present in pulmonary arteries are PDE III, which is cAMP-dependent and PDE V, which is cGMP-dependent [Rabe et al, 1994]. In addition, both milrinone and zaprinast dilate pulmonary arteries in various experimental models of pulmonary hypertension [Cohen et al, 1996, Hanasato et al, 1999, Hashiba et al, 2000, Jeffrey and Wanstall, 1998, McMahon et al, 1993, Nagamine et al, 2000]. PDE V is the most abundant cGMP hydrolyzing enzyme in the lung [Thomas et al, 1990], and is believed to limit the vasodilator effects of cGMP-mediated vasoactive substances such as nitric oxide. In addition, levels of PDE V increase in pulmonary hypertension [Black et al, 2001], inhibition PDE V has antiproliferative effects in human pulmonary artery cells [Wharton et al, 2005], and sildenafil improves hemodynamics and exercise capacity in patients with symptomatic pulmonary arterial hypertension [Galie et al, 2005]. Thus, PDE V is an attractive target to immobilize to allow for the maintenance of normal pulmonary vascular tone. In further support of this theory, the selective PDE V inhibitor sildenafil has been shown to attenuate the increase in pulmonary arterial pressure and vascular remodeling that occurs during chronic exposure to hypoxia [Sebkhi et al, 2003].

K⁺ channels play an essential role in regulating resting membrane potential and contractile tone of vascular smooth muscle [Archer et al, 1998, Barnes and Liu, 1995, Nelson and Quayle, 1995, Yuan, 1995, Yuan et al, 1998]. 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, including pulmonary arteries [Nelson and Quayle, 1995]. Activation of BK_{Ca} channels leads to membrane hyperpolarization and causes vasodilation, whereas BK_{Ca} channel inhibition causes membrane depolarization and vasoconstriction, which has been implicated in the development of pulmonary hypertension [Post et al, 1992]. The role of PDE III and PDE V in modulating BK_{Ca} channel activity in FHR PASMCs may involve PKG-mediated signaling, as we have recently demonstrated a novel pathway whereby cAMP-elevating vasodilators open BK_{Ca} channels via “cross-talk” activation of PKG independent of PKA in FHR PASMC [Barman et al, 2003].

The study of pulmonary hypertension has been problematic because of the lack of relevant animal models. In addition, it is difficult in many experimental models to separate the vascular changes that may be pathogenic from the changes that occur secondary to the hypertension [Ashmore et al, 1991]. The FHR strain has been widely used to study genetic risk factors for the development of pulmonary hypertension [Sato et al, 1992], which is not associated with differences in blood gas tension, polycythemia, or parenchymal lung disease [Zamora et al, 1996]. Unlike other rat strains, the FHR develops severe pulmonary hypertension that is age dependent, a phenomenon accelerated under mild hypoxic conditions [Sato et al, 1992]. Evidence also suggests that the genetic locus for the hypertensive condition is PH1 on chromosome 1 [Stelzner et al, 1997].

In conclusion, the results of the present study show that the non-selective phosphodiesterase (PDE) inhibitor isobutylmethylxanthine (IBMX), the specific PDE III inhibitor milrinone, and the specific PDE V inhibitor zaprinast reverse the inhibitory effect of thymeleatoxin on BK_{Ca} channels in FHR PASMC, and inhibit the vasoconstrictor response to PMA in FHR isolated pulmonary arteries. In addition, PMA significantly increases both PDE III and PDE V activity in FHR PASMC, which suggests a causal relationship between PKC and phosphodiesterase signaling in hypertensive pulmonary arterial smooth muscle. These results also suggest that the use of PKC inhibitors may be an approach to decrease PDE activity, which may contribute to the development of new modalities to treat pulmonary hypertension.

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Figure 1.

PKC activation inhibits the effect of forskolin (FSK) on BKC_a channel activity as measured by open probability (NPo) in FHR PASMC. 10 μ M FSK opens BKC_a channels, which is blocked by 100nM thymeleatoxin (THX). Recordings are from the same cell-attached patches (n = 4 for each group). * Significantly different from FSK, $P < 0.05$ (n = 4).

Figure 2.

100 μ M IBMX (non-specific PDE inhibitor) reverses 100nM thymeleatoxin (THX) inhibition of 10 μ M forskolin (FSK)-induced BKCa channel activity (right panel). IBMX and THX alone have no effect on BKCa channel activity (left panel). Channel openings are upward deflections from baseline (dotted line, closed) state. Recordings are from the same cell-attached patches ($n = 4$ for each group).

Figure 3.

Phosphodiesterase (PDE) inhibition by milrinone reverses the effect of PKC on BKCa channel activity in FHR PASMC. 10 μ M milrinone (cAMP PDE) reverses 100nM thymeleatoxin (THX) inhibition of 10 μ M forskolin (FSK)-induced BKCa channel activity (right panel). Milrinone and THX alone have no effect on BKCa channel activity (left panel). Channel openings are upward deflections from baseline (dotted line, closed) state. Recordings are from the same cell-attached patches (n = 4 for each group).

Figure 4.

Phosphodiesterase (PDE) inhibition by zaprinast reverses the effect of PKC on BKCa channel activity in FHR PASMC. 10 μ M zaprinast (cGMP PDE) reverses 100nM thymeleatoxin (THX) inhibition of 10 μ M forskolin (FSK)-induced BKCa channel activity (right panel). Zaprinast and THX alone have little effect on BKCa channel activity (left panel). Channel openings are upward deflections from baseline (dotted line, closed) state. Recordings are from the same cell-attached patches ($n = 4$ for each group).

Figure 5.

100nM thymeleatoxin (THX) inhibits forskolin (FSK)-induced BKCa channel open probability (NPo). THX alone has no effect on BKCa channel activity, and subsequent incision of the patch into an inside-out configuration and raising cytoplasmic $[Ca^{2+}]$ produces an immediate increase in BKCa channel NPo in the presence of both THX and FSK, indicating the continued BKCa channel viability of these cells.

Figure 6.

IBMX inhibits the vasoconstrictor effect of phorbol myristate acetate (PMA) in isolated FHR pulmonary arteries. Treatment with PMA (100nM) for 30 min increases vessel tension by approximately 160%, which is significantly attenuated by pretreatment with 100 μ M IBMX for 30 min. * Significantly different from PMA, $P < 0.05$ ($n = 4$).

Figure 7.

Milrinone inhibits the vasoconstrictor effect of phorbol myristate acetate (PMA) in isolated FHR pulmonary arteries. Treatment with PMA (100nM) for 30 min increases vessel tension by approximately 150%, which is significantly attenuated by pretreatment with 10 μ M milrinone for 30 min. * Significantly different from PMA, $P < 0.05$ ($n = 4$).

Figure 8.

Zaprinast inhibits the vasoconstrictor effect of phorbol myristate acetate (PMA) in isolated FHR pulmonary arteries. Treatment with PMA (100nM) for 30 min increases vessel tension by approximately 175%, which is significantly attenuated by pretreatment with 10 μ M zaprinast for 30 min. * Significantly different from PMA, $P < 0.05$ ($n = 4$).

Figure 9.

Activation of PKC increases PDE activity in FHR PASM. Assay of PDE III and PDE V activity in FHR pulmonary arterial smooth muscle shows that treatment with 100nM PMA for 30 minutes increases PDE III, and PDE V activity. * Significantly different from baseline, $P < 0.05$ ($n = 4$ for each group).