Inhibition of voltage-gated K⁺ currents by endothelin-1 in human pulmonary arterial myocytes

LARISSA A. SHIMODA, J. T. SYLVESTER, GREGORY M. BOOTH, TENILLE H. SHIMODA, SONYA MEEKER, BRADLEY J. UNDEM, AND JAMES S. K. SHAM
Divisions of Pulmonary and Critical Care Medicine and Allergy and Clinical Immunology, Johns Hopkins School of Medicine, Baltimore, Maryland 21224

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ENDOTHELIN (ET)-1 is a 21-amino acid peptide secreted by the vascular endothelium. After its release from the endothelium, ET-1 contracts smooth muscle by binding to either type A or type B endothelin receptors, both of which are abundantly present in the pulmonary vasculature (21). ET-1-induced constriction is well characterized in the pulmonary vasculature of the cat, rat, guinea pig, dog, and rabbit (5, 7, 17, 19, 22, 23, 25, 34). Recently, ET-1 was found to cause constriction in human pulmonary arteries (6, 15, 27, 31). Understanding the mechanisms by which ET-1 causes constriction in the human pulmonary vasculature is important, since inhibitors of these channels, but not KCa or KATP channels, cause depolarization and increased [Ca²⁺]i (2, 35, 38). In cultured human PASMCs, ET-1 can activate and inhibit KCa channels, depending on the concentration (31). Interestingly, ET-1 was found to have no effect on Kv channels in these cells (31). In culture, however, K⁺ channel and ET receptor distribution might be altered; thus, the effect of ET-1 on K⁺ channels in cultured PASMCs may not accurately reflect the effects of ET-1 on K⁺ channels in freshly isolated cells. Therefore, in this study, we used human pulmonary arterial segments and freshly isolated human PASMCs to determine whether ET-1 inhibits Kv channels.
determine whether the effect of ET-1 on \( K_v \) channels is PKC dependent.

**METHODS**

**Tissue Preparation**

Human lung tissue was obtained from 18 anonymous organ donors (supplied by the International Institute for the Advancement of Medicine, Exton, PA, or the Anatomical Gift Foundation, Woodbine, GA). Organ donor specimens were mainly obtained from victims of head trauma or cerebral vascular accidents. The donors were men (10) and women (8) with an average age of 28 ± 3 yr (range 10–57 yr). The lungs were macroscopically normal; lungs from donors with documented pulmonary pathology were not used. Donor organs were placed in cooled (4°C) MEM and transferred to the laboratory within 24 h. Upon arrival, tissues were immediately placed in cold physiological saline solution (PSS) containing (in mM) 130 NaCl, 5 KCl, 1.2 MgCl₂, 1.5 CaCl₂, 10 HEPES, and 10 glucose, with pH adjusted to 7.4 with NaOH.

**Isolation of PASMCs**

Single smooth muscle cells were obtained using methods previously described (35). Briefly, intrapulmonary arteries (800–2,000 \( \mu \)m OD) from the lower right lobe were dissected free of connective tissue and opened, and the lumen was gently scraped with a cotton swab to remove the endothelial cells. The arteries were placed in cold PSS for 30 min and then transferred to reduced Ca\(^{2+}\) PSS (20 \( \mu \)M CaCl₂) at room temperature for 20 min. The tissue was enzymatically digested for 20 min at 37°C in reduced Ca\(^{2+}\) PSS containing 5.5 mg/ml collagenase, 0.6 mg/ml papain, 2 mg/ml BSA, and 1 mM dithiothreitol. After digestion, single smooth muscle cells were dispersed by gentle titration with a wide-bore transfer pipette in Ca\(^{2+}\)-free PSS. The cell suspension was transferred to glass cover slips for study.

**Membrane Current Measurements**

Myocytes were continuously superfused with PSS. Membrane currents were measured using the whole cell patch-clamp technique. Patch pipettes (3–5 M\( \Omega \)) were filled with an internal solution containing (in mM) 35 KCl, 90 potassium gluconate, 10 NaCl, and 10 HEPES, with pH adjusted to 7.2 with KOH. GTP (0.5 mM) was added to provide substrate for signal transduction pathways, and Mg-ATP (5 mM) was included to inhibit KATP channels and provide substrate for metabolic pathways, and Mg-ATP (5 mM) was added. The internal solution contained 5.5 M KCl in rings subjected to denudation were required to be at least 5 g in 1-g steps over a period of 45 min. Preliminary experiments revealed that contractile responses to KCl (80 mM) were maximal at this resting tension. Arteries were exposed to 80 mM KCl to establish viability and maximum contraction and to phenylephrine (3 × 10⁻⁷ M) followed by ACh (10⁻⁶ M) to verify disruption of endothelial integrity. Vessels that did not exhibit an increase in tension ≥2 g in response to KCl or dilated by >10% in response to ACh were discarded. To confirm that endothelial denudation did not seriously damage smooth muscle, contractile responses to KCl in rings subjected to denudation were required to be ≥80% of responses measured in control rings with intact endothelium.

**Experimental Protocols**

Identification of \( K^+ \) currents in freshly isolated human pulmonary arterial myocytes. Membrane currents were elicited by applying an 800-ms depolarizing pulse from a holding potential of −60 mV to test potentials ranging from −50 to +60 mV in 10-mV increments before and 3–4 min after application of ET-1 (10⁻⁸ M). The measurements were made under control conditions, 3–4 min after applying charybdotoxin (ChTX; 100 nM), and 3–4 min after subsequent application of 4-aminopyridine (4-AP; 10 mM). Whole cell currents recorded in the presence of ChTX were further characterized by analyzing the time course of current inactivation, as described previously (35, 38), with the following biexponential equation: 

\[
I(t) = A_0 + A_1 e^{-t/\tau_1} + A_2 e^{-t/\tau_2},
\]

where \( I(t) \) is the current at time \( t \), \( A_0 \), \( A_1 \), and \( A_2 \) are the amplitudes of the nonactivating, rapidly inactivating, and slowly inactivating components, respectively, and \( \tau_1 \) and \( \tau_2 \) are the time constants of the rapidly and slowly inactivating components, respectively.

**Effect of ET-1 on \( K_v \) current.** To study the effect of ET-1 on the \( K_v \) current, experiments were performed in the presence of ChTX (100 nM) to inhibit \( K_{Ca} \) currents. The effect of ET-1 on \( K_v \) current was determined by measuring peak and steady-state (at 700–800 ms) membrane currents elicited by depolarizing pulses of 800 ms from −60 to +40 mV in 10-mV increments before and 3–4 min after application of ET-1 (10⁻⁸ M).

**Role of PKC in the effect of ET-1 on \( K_v \) current.** The involvement of PKC activation was examined by comparing the effects of ET-1 (10⁻⁸ M) on \( K_v \) currents generated in PASMCs before and 5 min after exposure to staurosporine (1 nM), a PKC inhibitor. We have previously demonstrated that this concentration of staurosporine is sufficient to inhibit ET-1-induced inhibition of \( K_v \) current in rat PASMCs (35).
Effect of \(K^+\) channel inhibition on pulmonary arteries. To determine whether inhibition of \(K^+\) channels could cause contraction in human pulmonary arteries, we examined the effect of 4-AP, a \(K^+\) channel inhibitor, on baseline tension. Endothelium-denuded pulmonary arterial segments were adjusted to a baseline tension of 5 g and challenged with 1 mM 4-AP. When the increase in tension had stabilized, the arteries were then challenged with a higher concentration of 4-AP (5 mM). Contraction is expressed as maximum tension generated at each concentration normalized to the maximum tension induced by 80 mM KCl.

Role of PKC in ET-1-induced contraction. To verify the ability of ET-1 to constrict human pulmonary vascular smooth muscle and evaluate whether activation of PKC was involved in the generation of tension in response to ET-1, endothelium-denuded pulmonary arterial segments were mounted for isometric tension recording. One pair of vessel segments per lung was challenged with increasing concentrations of ET-1 (10\(^{-10}\) to 3 \(\times\) 10\(^{-8}\) M) in the absence or presence of staurosporine (100 nM) or GF-109203X (GFX; 100 nM), a specific PKC inhibitor. Contraction was expressed as maximum tension generated at each concentration normalized to the maximum tension induced by 80 mM KCl.

Drugs and Chemicals

ET-1 and ChTX were obtained from American Peptides (Sunnyvale, CA). GFX was obtained from Calbiochem (La Jolla, CA). Stauroporine, 4-AP, and all other chemicals were obtained from Sigma (St. Louis, MO). Stock solutions of ET-1 (10\(^{-5}\) M) and ChTX (10\(^{-4}\) M) were made up in distilled water, divided into aliquots, and kept frozen at −20°C. On the day of experiment, the solutions were diluted as needed with PSS. Stock solutions of stauroporine (10\(^{-2}\) M), GFX (10\(^{-2}\) M), and 4-AP (10\(^{-1}\) M) were made fresh on the day of study. 4-AP was made in PSS, and the pH was adjusted to 7.4 with HCl.

Statistical Analysis

Statistical significance was determined by the Student’s \(t\)-test (paired and unpaired as applicable) and two-way ANOVA with repeated measures. A \(P\) value < 0.05 was accepted as significant. In the text, data are expressed as means ± SE, where \(n\) refers to the number of cells or arteries tested. Experiments were performed on cells from at least three different lungs or on arterial pairs from different lungs.

RESULTS

Identification of \(K^+\) Current in Freshly Isolated Human PASMCs

Average cell capacitance of freshly isolated human PASMCs was 25.0 ± 1.66 pF (\(n = 21\)), close to values reported in cultured human PASMCs (30, 31). Outward \(K^+\) currents from human intrapulmonary arterial smooth muscle cells were measured using the whole cell voltage-clamp technique. The outward currents could be separated into two major components. One component was inhibited by ChTX (100 nM), a \(K\text{Ca}\) channel antagonist. This component of current represented 17.7% of the peak and 40% of the steady-state current at +20 mV and 18.4% of the peak and 38.5% of the steady-state current at +60 mV. After inhibition of \(K\text{Ca}\) channels, a second component of current was observed that exhibited rapid, voltage-dependent activation and time-dependent inactivation (Fig. 1). The current was activated at potentials positive to −30 mV. 4-AP (10 mM) inhibited 85% (both the peak and steady-state current) of the remaining ChTX-insensitive current at +20 mV. These characteristics are consistent with the \(K^+\) current described in previous studies (35, 38).

The inactivation kinetics of the \(K^+\) current were further quantified by fitting the descending portions of the \(K^+\) current, as described above. This approach separated the whole cell \(K^+\) current into rapidly and slowly inactivating and nonactivating components (Table 1). All three components contributed similarly to the total current, and all were sensitive to 4-AP.

### Table 1. Effect of ET-1 on \(K^+\) current amplitudes and time constants of inactivation

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ET-1</th>
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<tbody>
<tr>
<td>(A_0), pA</td>
<td>57.2 ± 11.5</td>
<td>36.6 ± 8.1*</td>
</tr>
<tr>
<td>(A_1), pA</td>
<td>52.7 ± 10.0</td>
<td>40.1 ± 9.9*</td>
</tr>
<tr>
<td>(A_2), pA</td>
<td>34.4 ± 6.3</td>
<td>26.8 ± 6.1</td>
</tr>
<tr>
<td>(\tau_i), ms</td>
<td>13.0 ± 1.3</td>
<td>8.5 ± 1.8*</td>
</tr>
<tr>
<td>(\tau_o), ms</td>
<td>81.2 ± 11.8</td>
<td>60.3 ± 14.2</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. ET-1, endothelin-1 (10\(^{-8}\) M); \(K^+\), voltage-dependent \(K^+\); \(A_0\), steady-state amplitude; \(A_1\), amplitude of rapidly inactivating component; \(A_2\), amplitude of slowly inactivating component; \(\tau_i\), time constant of rapidly inactivating component; \(\tau_o\), time constant of slowly inactivating component. *Significant difference from control value, \(P < 0.05\) by paired Student’s \(t\)-test.
Effect of ET-1 on \( K_v \) Currents

After addition of 100 nM ChTX to inhibit \( K_v \) channels, application of ET-1 (10\(^{-8}\) M; \( n = 12 \)) caused a significant inhibition of \( K_v \) current (Fig. 2). The current measured in the presence of ET-1 was reduced at all test potentials positive to \(-30\) mV. At \( +20 \) mV, ET-1 decreased peak \( K_v \) current density by 29.3\%, from 5.8 ± 1.5 to 4.1 ± 0.9 pA/pF, as represented in the downward shift in the current-voltage (I-V) relationship. ET-1 inhibited the steady-state portion of the \( K_v \) current by 48.8\%, from 1.76 ± 0.4 to 0.9 ± 0.2 pA/pF. The effect of ET-1 on \( K_v \) currents in the human PASMCs was similar in magnitude to that we previously observed in PASMCs from rats (35).

Analysis of the \( K_v \) inactivation kinetics indicated that ET-1 significantly reduced the time constant of the rapidly inactivating component and the amplitudes of the rapidly inactivating and noninactivating components (Table 1). Although ET-1 also appeared to reduce the time constant of the slowly inactivating component and the amplitude of the slowly inactivating component, the decrease in these values did not reach statistical significance. The effect of ET-1 on the inactivation kinetics of the \( K_v \) current is shown by superimposing the peak normalized \( K_v \) current in the absence and presence of ET-1 (Fig. 3), showing significant enhancement of inactivation and reduction in the steady-state current.

Role of PKC Activation in ET-1-Induced Inhibition of \( K_v \) Current

We have previously shown that PKC agonists inhibit the \( K_v \) current and that ET-1-induced inhibition of the \( K_v \) current in rat PASMCs requires activation of PKC (35). To determine the role of PKC activation on the effect of ET-1 on \( K_v \) in human PASMCs, we pretreated PASMCs with staurosporine (10\(^{-9}\) M), a widely used nonspecific PKC inhibitor, before ET-1 (10\(^{-8}\) M) challenge. Exposure to staurosporine had no significant effect on \( K_v \) current (3.9 ± 0.8 to 3.6 ± 0.9 and 2.3 ± 0.8 to 2.1 ± 0.9 pA/pF for peak and steady-state current at \(+20\) mV, respectively; \( n = 6 \)). In the presence of staurosporine, ET-1 had no effect on \( K_v \) current in human PASMCs (3.5 ± 0.8 and 2.0 ± 0.8 pA/pF for peak and steady-state currents at \(+20\) mV, respectively), causing no downward shift in the \( K_v \) current I-V relationships (Fig. 4). ET-1 also failed to decrease \( K_v \) current inactivation kinetics (Table 2), and normalized \( K_v \) currents in the presence of staurosporine before and after application of ET-1 were identical (Fig. 5).

Effect of 4-AP on Isolated Human Pulmonary Arteries

To determine whether an inhibition of \( K_v \) channels, as occurs with ET-1, can influence tone in human pulmonary arteries, endothelium-denuded arterial segments were adjusted to a stable baseline tension of 5 g. Addition of 1 mM 4-AP caused a significant increase in tension that reached a plateau at 16.9 ± 1.2\% of the maximum tension induced by 80 mM KCl (10.0 ± 2.6 g; \( n = 3 \)). Subsequent challenge with 5 mM 4-AP caused an additional sustained increase in tension to 102.1 ± 8.8\% of the maximum KCl tension (Fig. 6).
Role of PKC in ET-1-Induced Contraction in Isolated Human Pulmonary Arteries

To verify the contractile effect of ET-1 on the human pulmonary vasculature, arterial segments were challenged with exogenous ET-1 while isometric tension was monitored. Application of increasing concentrations of ET-1 (10^{-10} to 10^{-8} M) caused concentration-dependent contraction of endothelium-denuded human pulmonary arteries (n = 4; Fig. 7), reaching a maximum of 118.3 ± 10.3% of the tension induced by KCl at 3 × 10^{-8} M ET-1. In vessels pretreated for 15 min with staurosporine (10^{-9} M), the ET-1-induced increase in tension was blunted, although the difference for the entire concentration-response curve did not reach significance with ANOVA (P = 0.07). However, contrast analysis indicated significant differences in the presence and absence of staurosporine at 3 × 10^{-9} and 10^{-8} M ET-1 (P < 0.01), where contraction in response to ET-1, expressed as a fraction of maximum tension induced by KCl, was reduced from 0.49 ± 0.05 to 0.16 ± 0.05 at 3 × 10^{-9} M ET-1 and from 1.02 ± 0.2 to 0.73 ± 0.2 at 10^{-8} M ET-1 (n = 4). Because staurosporine can have nonselective effects, the effect of a putative selective PKC inhibitor, GFX (10^{-7} M; see Ref. 26), was also examined. At the concentration of ET-1 where inhibition by staurosporine was maximum (3 × 10^{-9} M), GFX reduced ET-1-induced contraction, expressed as a fraction of maximum KCl-induced tension, from 0.67 ± 0.09 to 0.37 ± 0.06 (n = 3), further supporting a role for PKC activation in ET-1-induced contraction (Fig. 7). Maximum tension induced by KCl, measured at the beginning of the experiment, was similar in all arteries tested: 7.2 ± 2.1 g in control

Table 2. Effect of ET-1 on K_v current amplitudes and time constants of inactivation in the presence of staurosporine

<table>
<thead>
<tr>
<th></th>
<th>Stauro</th>
<th>ET-1</th>
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<tbody>
<tr>
<td>A_0, pA</td>
<td>87.2 ± 24.7</td>
<td>85.9 ± 25.5</td>
</tr>
<tr>
<td>A_1, pA</td>
<td>39.0 ± 12.6</td>
<td>36.2 ± 10.5</td>
</tr>
<tr>
<td>A_2, pA</td>
<td>20.7 ± 3.0</td>
<td>23.8 ± 2.7</td>
</tr>
<tr>
<td>τ_1, ms</td>
<td>7.0 ± 1.4</td>
<td>9.7 ± 6.6</td>
</tr>
<tr>
<td>τ_2, ms</td>
<td>160.1 ± 63.2</td>
<td>129.2 ± 71.9</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. Stauro, staurosporine (10^{-9} M).

Fig. 4. Effect of the protein kinase C inhibitor staurosporine (Stauro; 10^{-9} M) on the response to ET-1 (10^{-8} M). A: representative traces demonstrating K_v currents measured under control conditions and in the presence of Stauro before and after exposure to ET-1. B and C: mean current-voltage relationship for peak (B) and steady-state (C) currents measured in the presence of Stauro before and after addition of ET-1 (n = 6 cells from 3 lungs). Measurements were made in the presence of 100 nM ChTX.

Fig. 5. Effect of ET-1 (10^{-8} M) on the rate of K_v current inactivation in the presence of Stauro (10^{-9} M). Measurements were made in the presence of 100 nM ChTX. Results were similar to those obtained in 4 other cells from 3 lungs.

Fig. 6. Effect of inhibition of K_v channels with 4-AP (1 and 5 mM) on isometric tension measured in human pulmonary arterial segments (n = 3). Resting tension was 5 g. Contraction is expressed as a fraction of maximum tension induced by KCl.

Fig. 7. Effect of inhibition of K_v channels with 4-AP (1 and 5 mM) on isometric tension measured in human pulmonary arterial segments (n = 3). Resting tension was 5 g. Contraction is expressed as a fraction of maximum tension induced by KCl.
arteries, 6.7 ± 2.0 g in arteries subsequently exposed to staurosporine, and 9.0 ± 1.5 g in arteries subsequently exposed to GFX.

DISCUSSION

In this study, we found that ET-1 constricts human pulmonary arteries and inhibits Kv currents in human intrapulmonary arterial smooth muscle cells. Both the inhibitory effect of ET-1 on Kv current and ET-1-induced contraction could be attenuated by pretreatment with staurosporine, suggesting involvement of a mechanism requiring activation of PKC.

Kv channels are a major regulator of membrane potential in pulmonary vascular smooth muscle, since inhibitors of Kv channels cause depolarization in rat PASMCs (2, 35, 38), increase [Ca^{2+}]_i in cultured human PASMCs (39), and increase tension in human pulmonary arteries (30). Kv channels have been pharmacologically identified in cultured human PASMCs (18, 30, 39); however, because K^+ channel expression and/or activity can be modified in culture (28), we verified the contribution of Kv channels to the whole cell outward K^+ current measured in freshly isolated human PASMCs. Under these conditions, Kv current, defined as that portion of the K^+ current sensitive to 4-AP but not to ChTX, comprised 57% of the total peak outward K^+ current and 38% of the total steady-state K^+ current. The Kv current in the freshly isolated human PASMCs consisted of at least three components (noninactivating, rapidly inactivating, and slowly inactivating). These components closely resemble the Kv current observed in PASMCs from the rat (35, 38) and may suggest that multiple Kv channel subtypes are present.

Peng et al. (31) demonstrated a dual effect of ET-1 on KCa currents in cultured human PASMCs, with ET-1 activating KCa current at low concentrations and inhibiting KCa current at high concentrations. In the present study, we found that ET-1 also inhibited Kv currents in human PASMCs. At 10^{-8} M, ET-1 caused a 29% decrease in the peak Kv current density and a 49% decrease in the steady-state portion of the current. The difference in magnitude of inhibition between the peak and steady-state portions of the Kv current suggested that ET-1 might have different effects on the three components of Kv current we had identified. Comparison of the inactivation kinetics of Kv current in the absence and presence of ET-1 indicated that, although the amplitude of all three components appeared to decrease after addition of ET-1, the decrease in the amplitude of the slowly inactivating component did not reach statistical significance. In addition to decreasing the amplitudes of the rapidly inactivating and noninactivating components, ET-1 also decreased the time constant of the rapidly inactivating component of the Kv current. These effects of ET-1 on Kv current kinetics in human PASMCs are qualitatively similar to those we previously reported in the rat (35).

The exact mechanism by which ET-1 inhibits Kv currents is unclear. Agonist-induced increases in [Ca^{2+}]_i inhibit Kv currents in vascular smooth muscle (33). Because ET-1 increases [Ca^{2+}]_i levels in rat PASMCs (4, 36), it is probable that an ET-1-induced rise in [Ca^{2+}]_i also occurs in human PASMCs. The inhibitory effect of ET-1 on Kv currents observed in the current study was not likely a secondary effect of increased [Ca^{2+}]_i, however, because our cells were dialyzed with BAPTA, a strong Ca^{2+} chelator that prevents changes in global [Ca^{2+}]_i. A role for PKC activation in ET-1-induced contractile responses has been established in several vascular beds, including...
the lung (5, 11), and studies from our laboratory and others indicate that PKC activation inhibits Kv currents in smooth muscle (1, 35). Furthermore, we have demonstrated in rat PASMCs that the inhibitory effect of ET-1 on Kv current requires activation of PKC secondary to activation of PLC (35). Consistent with these findings, the inhibitory effect of ET-1 on human PASMCs was abolished after pretreatment with staurosporine. The exact mechanisms involved in PKC-induced inhibition of the Kv current are not well understood and perhaps involve direct phosphorylation of Kv channels by PKC (32), indirect phosphorylation through second messengers that modify Kv channel activation (16), or PKC-dependent activation of β-subunits (20), which enhance Kv channel inactivation when associated with Kv channel α-subunits (9, 29). Further experiments will be required to elucidate the exact mechanism responsible for this phenomenon.

Application of exogenous ET-1 to endothelium-deleted human pulmonary arteries resulted in a concentration-dependent increase in isometric tension. The maximum response occurred at $10^{-8}$ M, consistent with results observed in the pulmonary vasculature of other species (7, 17, 22). We previously demonstrated that ET-1 initiates a complex series of events in rat PASMCs, including activation of PLC and PKC, inhibition of Kv currents, depolarization, activation of $Ca^{2+}$ influx, and $Ca^{2+}$ release from the sarcoplasmic reticulum (35, 36). An initiating step in the ET-1 contractile process in rat PASMCs appears to be PKC-dependent inhibition of Kv currents, since depolarization preceded the rise in $[Ca^{2+}]_i$ (36). In the present study, we found that 4-AP increased tension in human pulmonary arterial segments, verifying that modulation of Kv channels can regulate tone in this vascular bed. Moreover, pretreatment of arteries with PKC inhibitors, which prevented inhibition of Kv current by ET-1, significantly reduced the ability of ET-1 to increase tension, providing further evidence supporting the possibility of a role for PKC-dependent inhibition of Kv current in ET-1-induced contraction.

In summary, we found that ET-1 inhibits Kv channels and causes constriction in human pulmonary arteries. Furthermore, the effects of ET-1 were attenuated in the presence of PKC inhibitors. These findings suggest that ET-1 modulates pulmonary vascular reactivity in humans, in part via signal transduction pathways involving activation of PKC and inhibition of Kv currents. Moreover, the effects of ET-1 on Kv channels in PASMCs from rats and humans were qualitatively similar, and both appear to require activation of PKC, suggesting that PKC-dependent inhibition of Kv channels by ET-1 may be conserved among species.

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REFERENCES
2. Archer SL, Huang JM, Reeve HL, Hampel V, Tolarova S, Michelakis E, and Weir EK. Differential distribution of electrophysiologically distinct myocytes in conduit and resistance arteries. Furthermore, the effects of ET-1 were attenuated in the presence of PKC inhibitors. These findings suggest that ET-1 modulates pulmonary vascular reactivity in humans, in part via signal transduction pathways involving activation of PKC and inhibition of Kv currents. Moreover, the effects of ET-1 on Kv channels in PASMCs from rats and humans were qualitatively similar, and both appear to require activation of PKC, suggesting that PKC-dependent inhibition of Kv channels by ET-1 may be conserved among species.

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