Enhanced ET\textsubscript{A}-receptor-mediated inhibition of K\textsubscript{v} channels in hypoxic hypertensive rat pulmonary artery myocytes

KAI-XUN LI,1 BRIAN FOUTY,1 IVAN F. McMURTRY,1 AND DAVID M. RODMAN1,2

Departments of 1Medicine and 2Physiology and Biophysics, Cardiovascular Pulmonary Research Laboratory, University of Colorado Health Science Center, Denver, Colorado 80262

Li, Kai-Xun, Brian Fouty, Ivan F. McMurry, and David M. Rodman. Enhanced ET\textsubscript{A}-receptor-mediated inhibition of K\textsubscript{v} channels in hypoxic hypertensive rat pulmonary artery myocytes. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H363–H370, 1999.—Endothelin (ET)-1 has been implicated as a critical mediator in the pathogenesis of hypoxic pulmonary hypertension. We questioned whether, during exposure to chronic hypobaric hypoxia, rat pulmonary artery smooth muscle cells (PASMC) became sensitized to ET-1. Two effects of ET-1, inhibition of voltage-gated K\textsuperscript{+} (K\textsubscript{v}) channels and release of intracellular Ca\textsuperscript{2+}, were studied using whole cell patch clamp and single cell indo 1 fluorescence, respectively. In both normotensive and chronically hypoxic-hypertensive PASMC, ET-1 caused concentration-dependent inhibition of voltage-gated K\textsuperscript{+} current (I\textsubscript{K(v)}), with maximum inhibition of 12–18% seen at a concentration of 0.1–1 nM. Although the chronically hypoxic-hypertensive PASMC was no more susceptible to ET-1-mediated I\textsubscript{K(v)} inhibition, a switch in coupling between ET-1 and I\textsubscript{K(v)} from ET\textsubscript{A} to ET\textsubscript{B} receptors occurred. This switch in receptor coupling, combined with reduced I\textsubscript{K(v)} density and increased ET-1 production in the hypoxic rat lung, may help explain the ability of ET\textsubscript{A}-receptor blockers to attenuate the development of hypoxic pulmonary hypertension in vivo.

Keywords: pulmonary hypertension; ion channel; potassium channel; cytosolic calcium; vascular smooth muscle cell

PULMONARY ARTERY VASOCONSTRICTION and medial hypertrophy are cardinal manifestations of hypoxic pulmonary hypertension. Although significant progress has been made in understanding the signaling events that link chronic hypoxia to alterations in vascular tone and structure, important unresolved issues remain. Recent evidence suggests that the peptidergic vasoconstrictor endothelin (ET)-1 is an important mediator of the vascular changes associated with hypoxic pulmonary hypertension. Both steady-state mRNA for the pre-proET-1 transcript as well as lung levels of the mature peptide are elevated in hypoxic pulmonary hypertension (7). In addition, circulating levels of ET-1 are elevated in a variety of animal models of pulmonary hypertension, as well as humans with both primary and secondary pulmonary hypertension (1, 34). Importantly, pharmacological inhibition of ET-1-receptor activation ameliorates the development of pulmonary hypertension in chronically hypoxic rats, suggesting that the increased levels of ET-1 may be etiologic in the development of hypoxic pulmonary hypertension (4–6).

However, the mechanisms through which ET-1 affects pulmonary artery smooth muscle cell (PASMC) activation both in the normotensive and the hypoxic-hypertensive circulation have not been fully characterized. At least two mechanisms of action of ET-1 in PASMC exist, 1) inhibition of voltage-gated K\textsuperscript{+} (K\textsubscript{v}) channels and 2) inositol 1,4,5-trisphosphate-mediated Ca\textsuperscript{2+} release from intracellular stores (15). An important unresolved controversy is the relative contribution of ET\textsubscript{A} and ET\textsubscript{B} receptors to these downstream signaling events in the hypertensive PASMC. Previous investigators found that in normotensive resistance PASMC inhibition of K\textsubscript{v} channels was mediated by ET\textsubscript{B}-receptor activation (22). The membrane depolarization resulting from K\textsubscript{v}-channel inhibition primed the cells for subsequent hypoxia-induced Ca\textsuperscript{2+} influx (29). In contrast to the effect on K\textsubscript{v} channels, release of intracellular Ca\textsuperscript{2+} stores was mediated by activation of both ET\textsubscript{A} and ET\textsubscript{B} receptors (10). The relevance of these observations to the hypertensive pulmonary circulation is uncertain.

Although both K\textsubscript{v}-channel inhibition and release of intracellular Ca\textsuperscript{2+} stores could be important in the genesis of hypoxic pulmonary hypertension, data suggest that ET-receptor coupling to downstream signaling events may be altered in the hypertensive pulmonary circulation. McCulloch et al. (16) found that in the normotensive PASMC, ET-1 potency was increased after pharmacological inhibition of ET\textsubscript{A} receptors, suggesting a predominant inhibitory role for the ET\textsubscript{A} receptor. In contrast, in hypertensive PASMC, activation of the ET\textsubscript{A} receptor mediated pulmonary vasoconstriction, suggesting a switch from smooth muscle cell inhibition to activation via the ET\textsubscript{B} receptor (16). These studies were limited to isolated pulmonary artery rings and did not elucidate the cellular mechanisms of action of ET-1.

On the basis of this information, we hypothesized that in hypoxic-hypertensive pulmonary circulation a switch in receptor coupling between ET-1 and either K\textsubscript{v} channels or release of intracellular Ca\textsuperscript{2+} stores occurs, resulting in enhanced ET\textsubscript{A}-mediated activation of the PASMC. To evaluate this hypothesis we used whole cell patch clamp to measure the effect of ET-1 on macroscopic K\textsubscript{v} current in freshly dispersed PASMC isolated from resistance pulmonary arteries from normoxic and chronically hypoxic rats. We also measured the effects on cytosolic free Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]) using the Ca\textsuperscript{2+}-sensitive fluorophore indo 1. The relative contribution of ET\textsubscript{A} and ET\textsubscript{B} receptors was assessed using the receptor-selective blockers BQ-123 and BQ-788. Our findings are consistent with the hypothesis that there is unique coupling of ET receptors to K\textsubscript{v} channels and that this coupling is altered in hypoxic pulmonary

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

0363-6135/99 $5.00 Copyright © 1999 the American Physiological Society
hypertension. The net result of these alterations is enhanced ETA-mediated inhibition of Kv channels, which, in combination with increased ET-1 peptide, may contribute to membrane depolarization and subsequent activation of voltage-gated Ca\(^{2+}\) channels.

MATERIALS AND METHODS

Hypoxic rats. Male Sprague-Dawley rats (200–300 g) were placed in a hypobaric chamber for 3–4 wk. Air was exchanged and pressure was monitored continuously with a mercury altimeter gauge (17,000 ft above sea level altitude; Po\(_2\) = 76 Torr). We took the rats out of the chamber for 10 min three times per week to clean the cages and replenish food. Chronically hypoxic rats were studied after 3–4 wk of hypobaric hypoxia. Control rats were housed at Denver’s altitude with otherwise identical care.

Dispersed PASMC preparation. Single PASMC were obtained with the modified method of Archer et al. (2). Briefly, normoxic and hypoxic rats were anesthetized with intraperitoneal injection of pentobarbital sodium (50 mg/kg body wt). Lungs were quickly removed from chest en bloc and transferred into a beaker containing cold (4°C) physiological salt solution (PSS). The PSS was composed of (in mM) 135 NaCl, 5.5 KCl, 2 CaCl\(_2\), 1 MgCl\(_2\), 10 HEPEs, and 10 glucose pH was adjusted to 7.4 with 5 M NaOH. Distal third and fourth divisions of the intrapulmonary arteries were carefully isolated, cleaned of adventitia, and opened longitudinally. After 15- to 20-min recovery in cold nominally Ca\(^{2+}\)-free PSS, the arteries were incubated for 15 min in cold Ca\(^{2+}\)-free PSS digestion solution containing (in mg/ml) 1 papain, 0.75 dithiothreitol, 1 BSA, and 1 trypsin inhibitor (for fluorescence studies 20 µM CaCl\(_2\) was added), after which the digestion solution was heated to 37°C for 18 min. After digestion the tissues were thoroughly washed, and single PASMC were dispersed by gentle trituration with a fire-polished glass pipette. Drops of cell suspension were placed onto the perfusion chamber for electrophysiological or microfluorometric studies. All cells were studied within 60 min of dissociation.

Immunohistochemistry. Immunohistochemical studies were performed to identify cells expressing smooth muscle α-actin using methods previously described (21). Briefly, serial Formalin-fixed, paraformaldehyde-embedded sections (6 µm) were placed on poly-L-lysine-coated slides, deparaffinized in three changes of xylene, and rehydrated in 100, 95, and 75% ethanol alcohol. The dilution that yielded optimal specific staining was determined in pilot experiments. Mouse anti-rat smooth muscle cell α-actin primary antibody (1:100 dilution; Atlantic Antibody, Stillwater, MN) was used. Control experiments were performed using rabbit serum. Biotinylated anti-rabbit immunoglobulin (1:200 dilution; Vector Laboratories, Burlingame, CA) was applied for 30 min, followed by a 30-min incubation in a Vectastain avidin-biotin-alkaline phosphatase reagent. The substrate 3-amin-9-ethyl-carbazole yielded a red reaction product, and sections were counterstained in methyl green.

Measurements of membrane potential and I\(_{K(v)}\). Gently dispersed single PASMC were placed in the perfusion chamber on the stage of an Olympus CK-2 inverted microscope (Tokyo, Japan) equipped with a ×100 fluorescence objective lens. An ultraviolet light beam from a 75-W xenon arc lamp was filtered at 365 nm to excite indo 1. Emitted fluorescence signal was directed to a dichroic mirror. Transmitted light and reflected light were filtered at 480 and 405 nm, respectively, and measured by two separate photometers (PTI, Princeton, NJ). The emission ratio of 405 to 480 nm was calculated as an index of [Ca\(^{2+}\)]\(_i\); and stored in a Macintosh PowerPC computer (Apple, Cupertino, CA) using a 12-bit MacLab/8 interface (Analog Digital Instruments, Milford, MA). For calculating [Ca\(^{2+}\)]\(_i\) the following formula was used: [Ca\(^{2+}\)]\(_i\) = K\(_b\)(R - R\(_\text{min}\))/R\(_\text{max}\) - R, where K\(_b\) is the apparent dissociation constant of the Ca\(^{2+}\)-sensitive fluorophore indo 1 and is taken as 250 nM (8); R\(_\text{max}\) is the fluorescence ratio (R) for the Ca\(^{2+}\)-saturated dye; R\(_\text{min}\) is R for the Ca\(^{2+}\)-unbound dye; and R is the ratio of the 480-nm fluorescence obtained for the Ca\(^{2+}\)-free and Ca\(^{2+}\)-bound forms of the dye. Values for R\(_\text{max}\) and R\(_\text{min}\) were determined in vivo in PASMC after permeabilization with 25 µM ionomycin and exposure to either 10 mM Ca\(^{2+}\) or 10 mM EGTA (0 mM Ca\(^{2+}\)) solutions, respectively (31). In the experiments measuring the effect of Ca\(^{2+}\) influx on the Ca\(^{2+}\)-transient, a Ca\(^{2+}\)-free superfuse was used. All experiments were conducted at 32°C.

Chemicals. BQ-788 (gift from Banyu Pharmaceutical, Tsukuba, Japan) and indo 1-AM were dissolved in DMSO and diluted before use. BQ-123 (Banyu Pharmaceutical), ET-1 (Peptides International, Louisville, KY), iberiotoxin (Alomone Labs), and other chemicals obtained from Sigma (St. Louis, MO) were dissolved in deionized water and exposed to either 10 mM Ca\(^{2+}\) or 10 mM EGTA (0 mM Ca\(^{2+}\)) solutions, respectively (31). In the experiments measuring the effect of Ca\(^{2+}\) influx on the Ca\(^{2+}\)-transient, a Ca\(^{2+}\)-free superfuse was used. All experiments were conducted at 32°C.
RESULTS

Cell isolation and myosin staining. Myosin staining of sections of intrapulmonary artery revealed two morphologically distinct populations of medial smooth muscle cells. The majority of cells were cuboidal in shape and stained only weakly for $\alpha$-actin. Cells in the inner medial layer were spindle shaped and arranged circumferentially and exhibited intense $\alpha$-actin staining. Enzymatic dispersion produced a mixture of cuboidal cells and elongated, spindle-shaped cells. The latter cells, which appeared grossly similar to smooth muscle cells in the subintimal layer, also stained strongly positive for $\alpha$-actin and were used for all subsequent electrophysiological studies (Fig. 1).

Passive electrical properties of PASMC. Using current-clamp technique and a ramp protocol, we measured RMP, whole cell input resistance, and capacitance in both normotensive (NT) and hypertensive (HT) PASMC. We found that the RMP was depolarized in HT PASMC [NT: $-48.7 \pm 1.5$ mV ($n = 35$); HT: $-43.2 \pm 1.5$ mV ($n = 28$), $P < 0.01$]. In contrast, whole cell capacitance and input resistance were the same in the PASMC from both NT and HT rats [capacitance: $12.7 \pm 0.7$ pF ($n = 39$) vs. $13.7 \pm 0.6$ pF ($n = 31$); input resistance: $4.7 \pm 0.5$ G$\Omega$ ($n = 39$) vs. $4.0 \pm 0.6$ G$\Omega$ ($n = 31$); $P = $ not significant (NS)].

Effect of chronic hypoxia on $I_{K(V)}$. $I_{K(V)}$ was studied under conditions that eliminated the other predominant outward currents: ATP-dependent and Ca$^{2+}$-dependent K$^+$ currents [$I_{K(Ca)}$]. To eliminate contamination from these currents, 5 mM ATP (to inactivate ATP-dependent K$^+$ channels) and 10 mM BAPTA (to chelate cytosolic free Ca$^{2+}$) were included in the pipette solution. In addition, Ca$^{2+}$ was omitted in the bath solution to minimize the possibility that Ca$^{2+}$ influx would activate $I_{K(Ca)}$. Membrane potential was held at $-80$ mV, and a family of depolarizing pulses was applied from $-60$ to $+60$ mV in 10-mV increments. Figure 2A shows a representative example of outward currents from a NT PASMC under these conditions. The current was evoked at a membrane potential more negative than $-30$ mV after a short period of delay and reached a steady state with a slow inactivation. Application of 100 nM iberiotoxin (a specific Ca$^{2+}$-dependent K$^+$ channel blocker) reduced K$^+$ current ($I_K$) by $2.3 \pm 1.4\%$ (3 cells from 3 rats), with no significant change in $I_{K(V)}$ threshold.

Fig. 2. Voltage-gated K$^+$ current [$I_{K(V)}$] from normotensive (NT) and hypertensive (HT) pulmonary artery smooth muscle cells (PASMC). Family of representative $I_{K(V)}$ records by depolarizing cells from $-60$ to $+60$ mV in 10-mV increments for 400 ms from NT (A) and HT (B) PASMC. C: current-voltage (I-V) curve generated from NT (●) and HT (○) PASMC. I-V curve shows that there was no change in $I_{K(V)}$ threshold. D: maximum $I_{K(V)}$ density at $+60$ mV from both NT (filled bar) and HT (open bar) PASMC. $I_{K(V)}$ in HT PASMC was significantly reduced ($^*_P < 0.01$).
indicating that the $I_{K(Ca)}$ contribution to the total $I_K$ was negligible.

In chronically hypoxic rats, $I_{K(v)}$ was smaller than that in the NT rats, but the characteristics of $I_{K(v)}$ and its I-V relationship in HT PASMC were similar to those in NT PASMC. Figure 2B shows a representative example of outward currents from a HT PASMC. As the I-V relationship in Fig. 2C demonstrates, the activation voltage did not differ between NT and HT PASMC. However, as Fig. 2D shows, maximum current density was reduced by 30% in HT PASMC ($P < 0.01$).

Effect of ET-1 on $I_{K(v)}$ in NT and HT PASMC. To determine whether ET-1 affected $I_{K(v)}$ under our experimental conditions, we applied ET-1 ($10^{-12}$–$10^{-8}$ M) before measurement of outward current elicited by depolarization to +60 mV. Concentration-response tests in NT PASMC showed multiple concentration-dependent effects. At a very low concentration ($10^{-12}$ M) ET-1 increased $I_{K(v)}$ by 10% in two of five cells, had no effect in one cell, and decreased $I_{K(v)}$ by 5% in two cells. At higher concentrations suppression of $I_{K(v)}$ became the predominant effect. At the highest concentration used ($10^{-8}$ M) all cells responded with a decrease in $I_{K(v)}$. Representative examples are shown in Fig. 3, A and B. The effect of ET-1 was only partially reversible (<20%). Similarly, in HT PASMC, the $I_{K(v)}$ inhibition was also concentration dependent, with multiple effects at lower concentrations. At higher concentrations ($10^{-9}$ M and $10^{-8}$ M), all cells showed a $I_{K(v)}$ decrease. Despite a tendency for some cells to show a small increase in $I_{K(v)}$ at lower concentrations, the magnitude of the increase was significantly less than the magnitude of the inhibition in $I_{K(v)}$. Thus, when group means of the effect of ET-1 on $I_{K(v)}$ were calculated, the net effect was a decrease in $I_{K(v)}$ at all but the lowest concentration tested. There was a concentration-dependent decrease in $I_{K(v)}$ in both NT and HT PASMC. The inhibition was maximal at an ET-1 concentration of $10^{-10}$ M in normotensive PASMC (Fig. 3C). Although the magnitude of the inhibition in $I_{K(v)}$ tended to be greater at higher concentrations of ET-1 in HT versus NT PASMC, this trend did not achieve statistical significance.

To further characterize the $K^+$ current that was inhibited by ET-1, we tested the effect of the $K^+$-channel blocker 4-aminopyridine (4-AP; 5 mM) on the outward currents in NT PASMC. We found that after a steady $I_{K(v)}$ recording was obtained application of 4-AP reduced $I_{K(v)}$ by $39.5 \pm 5.8\%$ at +60 mV ($n = 4$, $P < 0.01$). Subsequent application of ET-1 ($10^{-8}$ M) did not further reduce this current (Fig. 4).

Effect of BQ-123 and BQ-788 on ET-1-mediated inhibition of $I_{K(v)}$. We tested the relative contributions of the ET$_A$ and ET$_B$ receptors to ET-1-mediated inhibition of $I_{K(v)}$ in PASMC using the ET$_A$-receptor-selective inhibitor BQ-123 and the ET$_B$-receptor-selective inhibitor BQ-788. As Fig. 5 demonstrates, in NT PASMC ET$_A$-receptor blockade had no effect on ET-1-mediated $I_{K(v)}$ inhibition, whereas ET$_B$-receptor blockade significantly reduced the effect. The combination of ET$_A$- and ET$_B$-receptor blockade completely eliminated the response.

Effect of ET-1 on $\left[Ca^{2+}\right]_i$ in NT and HT PASMC. We measured the effect of ET-1 on $\left[Ca^{2+}\right]_i$ in NT and HT PASMC using indo 1 fluorescence. Resting $\left[Ca^{2+}\right]_i$ did not differ between NT and HT PASMC ($81 \pm 11\, nM$ (19 cells from 6 rats) in NT PASMC; $88 \pm 8\, nM$ (17 cells from 6 rats) in HT PASMC; $P = \text{NS}$). We tested the $\left[Ca^{2+}\right]_i$ response to a range of concentrations of ET-1. At concentrations of $10^{-12}$–$10^{-10}$ M no response was detected. At a concentration of $10^{-9}$ M 14% cells (1 of 7 cells) responded, whereas at $10^{-8}$ M, the percentage of cells responding increased to 82% (9 of 11 cells). The response occurred within 60 s of addition of $10^{-8}$ M ET-1 and was characterized by an initial large $Ca^{2+}$ transient followed by a train of smaller transients. The peak response was greater in HT PASMC (NT: $730 \pm 100\, nM$ ($n = 14$ cells from 8 rats) vs. HT: $1,238 \pm 253\, nM$ ($n = 10$ cells from 5 rats); $P < 0.05$), and the pattern of the response tended to be more irregular in HT cells.
The magnitude of the initial transient was not reduced by removal of extracellular Ca\(^{2+}\). The magnitude of the initial transient was shorter (control: 115 ± 17 s; 0 Ca\(^{2+}\); 57 ± 9 s; P < 0.05), and the prolonged train of transients was eliminated in the absence of extracellular Ca\(^{2+}\) (Fig. 6C).

**DISCUSSION**

ET-1-mediated pulmonary artery vasoconstriction is a critical event in the genesis of hypoxic pulmonary hypertension. Our studies addressed mechanisms through which ET-1 activates the pulmonary artery myocyte, comparing cells from normotensive rats to those isolated from chronically hypoxic rats. We chose to study only cells from distal third and fourth branch pulmonary arteries because prior investigators have demonstrated diversity of ion channel activity along the pulmonary arterial tree (17). Cells isolated from these "distal" pulmonary arteries have been reported to have predominantly K\(_v\) channels and a robust intrinsic hypoxic response, suggesting that they more accurately model hypoxic vasoconstriction in the intact lung (30).

Even within these small pulmonary arteries there are multiple "phenotypes" of smooth muscle cells. Consistent with a prior report of Hislop and Reid (9), on histological sections we found there were two morphologies of cells in the media. In the inner media PASMC were elongated and arranged circumferentially and strongly expressed smooth muscle α-actin. In the remainder of the media, cells were more cuboidal and generally stained only weakly for α-actin. Freshly dispersed cells similarly displayed multiple morphologies. We found that elongated cells always displayed strongly positive α-actin immunoreactivity. In contrast, rounded or cuboidal cells variably expressed α-actin. Therefore, we restricted electrophysiological studies to the elongated cells, which likely represent a vasoconstrictor phenotype.

Passive electrical properties did not differ between NT and HT PASMC. This suggests that gross differences in cell surface area and membrane permeability did not exist. However, membrane potential was more depolarized in hypertensive cells. This is consistent with several prior reports using both patch clamp and microelectrodes in isolated cells and intact resistance arteries (14, 25). One notable exception is a report by Suzuki and Twarog (27), who found hyperpolarization...
in resistance pulmonary arteries from chronically hypoxic rats. However, it is not certain whether the endothelium was intact in that preparation, and subsequent studies of isolated cells have failed to confirm that observation. The patch-clamp technique is not an ideal method of measuring membrane potential, because dialysis of intracellular contents with the pipette solution may alter channel and pump activity. However, because all cells were studied under identical conditions, the difference between NT and HT PASMC was probably qualitatively accurate. Thus the observed depolarization would likely place HT PASMC closer to the activation threshold for voltage-gated Ca\(^{2+}\) channels and thus render them more susceptible to activation by concurrent depolarizing stimuli such as hypoxia or circulating vasoconstrictors.

We focused the remainder of our studies on the effect of ET-1. First, we tested whether ET-1 inhibited \(K_v\) channels. Interestingly, at very low concentrations ET-1 had variable, albeit modest, effects. At a concentration of 1 pM, approximately equal proportions of cells showed increased, decreased, or unaffected \(I_{K(v)}\). This suggests that there may be subtle diversity of electrophysiological behavior even within the morphologically homogeneous population of cells chosen for study. At higher concentrations, ET-1 inhibited \(I_{K(v)}\) in most cells. Inhibition of \(I_{K(v)}\) by ET-1 is consistent with prior reports in NT PASMC (23, 24). HT PASMC displayed similar behavior, although at concentrations \(<10^{-8}\) M the percentage of cells in which \(I_{K(v)}\) was suppressed by ET-1 was greater in HT than NT PASMC. \(I_{K(v)}\) was partially inhibited by 4-AP, and ET-1 had no additional inhibitory effect in the presence of 4-AP. The nature of 4-AP-resistant \(K_v\) channels is uncertain. Yuan et al. (32, 33) demonstrated that 4-AP has a similar inhibitory effect on oxygen-regulated \(K^+\) channels in PASMC. Although not conclusive proof, this suggests that ET-1 and hypoxia may act through a similar class of ion channel.

Turner and Kozlowski (29) reported studies of the effect of ET-1 on hypoxia-induced depolarization of PASMC. They found that there was synergy between the two in that subthreshold concentrations of ET-1 primed the cells to depolarize in response to hypoxia. They concluded that the central role for ET-1 in hypoxic vasoconstriction in vivo may be based on this electrophysiological synergy. Our findings that HT PASMC were depolarized relative to NT cells, in combination with similar susceptibility to ET-1-mediated \(K_v\) inhibition, suggests that the hypertensive pulmonary artery myocyte may be “primed” to respond to hypoxia through the dual mechanisms of intrinsic depolarization and enhanced pulmonary ET-1 production.

All known biological effects of ET-1 are mediated via ET receptors. Two receptor subtypes, \(\text{ET}_A\) and \(\text{ET}_B\), have been described on pulmonary artery myocytes. We therefore tested the role of each in coupling ET-1 to \(I_{K(v)}\). In NT PASMC we found that the \(\text{ET}_A\)-receptor inhibitor BQ-123 did not alter the ET-1 effect. In contrast, the \(\text{ET}_B\)-receptor inhibitor BQ-788 reduced the effect by \(\sim 50\%\). This is qualitatively similar to a prior report by Salter and Kozlowski (22), who found that in NT PASMC, ET-1 inhibited \(I_{K(v)}\) via the \(\text{ET}_B\) receptor. However, in our studies, the combination of BQ-788 and BQ-123 was more effective than BQ-788 alone, completely eliminating the effect of ET-1. Although the explanation for this finding is uncertain, it suggests that there is some form of synergy between \(\text{ET}_A\) and \(\text{ET}_B\) receptors such that coupling of \(\text{ET}_A\) receptors to \(K_v\) channels can only occur in the presence of \(\text{ET}_B\) activation. Similar ET receptor cross talk was found in a study by Micklely et al. (18) of ET-1 vasoconstriction in intact rat mesenteric arterioles, suggesting that this may be a generalized phenomenon in the vasculature.

Effects of ET-receptor inhibition differed in HT PASMC. Although BQ-123 alone had no effect on NT PASMC, it reduced ET-1-mediated \(I_{K(v)}\) inhibition by 63% in HT cells. Again, the combination of BQ-123 and BQ-788 completely eliminated the response. Thus it appears that activation of the \(\text{ET}_A\) receptor alone becomes sufficient to reduce \(I_{K(v)}\) in HT PASMC. \(I_{K(v)}\) inhibition was not caused by release of intracellular Ca\(^{2+}\) stores, because the use of 10 mM BAPTA in the internal pipette solution, in combination with low extracellular Ca\(^{2+}\), would have prevented calcium ions from reaching the \(K_v\) channel. The increased efficacy of \(\text{ET}_A\)-receptor activation is consistent with a prior report from McCulloch et al. (16); they found that chronic hypoxia potentiated \(\text{ET}_A\)-receptor-mediated vasoconstriction in rat intrapulmonary arteries. It is also consistent with the finding of Li et al. (13) that there was increased \(\text{ET}_A\), but not \(\text{ET}_B\), receptor expression in hypoxic rat lung.

We wondered whether the pattern of receptor coupling to \(K_v\) channels was similar to another important mechanism of smooth muscle cell activation, release of intracellular Ca\(^{2+}\) stores. Release of intracellular Ca\(^{2+}\) was measured using the Ca\(^{2+}\)-sensitive fluorophore indo 1. We confirmed that the initial large Ca\(^{2+}\) transient was caused by release of intracellular stores by testing the effect of removal of extracellular Ca\(^{2+}\). Under the latter condition the peak increase in \([Ca^{2+}]_i\) was not reduced from that observed with 2 mM extracellular Ca\(^{2+}\), although the duration was attenuated and subsequent regenerative spikes in \([Ca^{2+}]_i\) were eliminated. These results are similar to multiple prior reports and validate the use of initial peak height as an index of release of intracellular Ca\(^{2+}\) stores.

We found that, in contrast to the effect on \(K_v\) channels, either BQ-123 or BQ-788 alone was sufficient to inhibit the Ca\(^{2+}\) transient in either NT or HT PASMC. Thus it appears that in PASMC, simultaneous activation of both \(\text{ET}_A\) and \(\text{ET}_B\) receptors is required for this signal transduction pathway. This finding differs somewhat from that reported by Hyvelin et al. (10). In that earlier study, BQ-123 completely abolished the Ca\(^{2+}\) transient in main pulmonary artery myocytes and in 21% of intrapulmonary myocytes, whereas BQ-788 had no effect on main pulmonary myocytes but inhibited the transient in 70% of intrapulmonary myocytes. The principal difference between our result and theirs was
that we found BQ-123 to have significantly more efficacy in intrapulmonary myocytes. Whether this discrepancy represents a strain difference (Sprague-Dawley vs. Wistar rats) or was caused by other methodological differences (such as restriction of our studies to a morphologically distinct subset of PASMC) is uncertain.

We found that the requirement for simultaneous activation of both subtypes of ET receptor to stimulate the \([Ca^{2+}]_i\) transient did not differ between NT and HT cells. Therefore, our data suggest that coupling of ET receptors to intracellular \(Ca^{2+}\) stores utilizes a different signal transduction pathway than does coupling to \(K_v\) channels. Another line of evidence supporting that hypothesis was analysis of the threshold response. The threshold for inhibition of \(K_v\) channels by ET-1 was 1–10 pM. In contrast, release of intracellular \(Ca^{2+}\) stores required 1–10 nM ET-1. Thus it appears that a more sensitive signaling pathway is utilized for coupling of ET receptors to \(K_v\) channels in PASMC.

These findings may help clarify the signaling mechanisms utilized in the hypertensive pulmonary circulation, and they could have relevance to prior observations in the intact pulmonary circulation. We previously reported (19) that in the chronically hypoxic rat lung, inhibition of nitric oxide synthase by \(N^G\)-nitro-L-arginine (L-NNA) unmasked vasoconstrictor tone. In the isolated, PSS-perfused chronically hypoxic rat lung, L-NNA vasoconstriction could be eliminated by removal of extracellular \(Ca^{2+}\) or by simultaneous blockade of low- and high-threshold voltage-gated \(Ca^{2+}\) channels, consistent with PASMC membrane depolarization (20). L-NNA vasoconstriction was also reduced ~60% by ET\(_A\)-receptor blockade and eliminated by simultaneous inhibition of both ET\(_A\) and ET\(_B\) receptors, suggesting that ET-1 was stimulating the membrane depolarization (19). That pattern of inhibition, partial reduction with BQ-123 and complete inhibition with the combination of BQ-123 and BQ-788, is nearly identical to the pattern we found for ET-1-mediated inhibition of \(I_{K(v)}\) in hypertensive PASMC. We cannot rule out the possibility that multiple mechanisms contribute to ET-1-mediated membrane depolarization in the hypertensive pulmonary circulation in vivo. In addition to inhibition of \(I_{K(v)}\), ET-1 may also activate \(Cl^-\) conductance through release of intracellular \(Ca^{2+}\) stores (3).

However, our results suggest the latter event cannot be the sole mechanism of depolarization, because it would be prevented by either ET\(_A\)- or ET\(_B\)-receptor blockade.

Our findings may also help to explain another paradox. Multiple investigators have found that ET\(_A\)-receptor blockade reduces pulmonary artery pressure in chronically hypoxic rats by 60–100% (4–6). Inhibition of L-type \(Ca^{2+}\) channels has a similar effect, again suggesting that the pulmonary vasoconstriction stimulated by ET-1 in chronically hypoxic rats is mediated by membrane depolarization and activation of voltage-gated \(Ca^{2+}\) channels. In vivo, the circulating concentration of ET-1 in chronically hypoxic animals and humans is in the range of 3–5 nM (1, 7). Our studies suggest that at that concentration maximum inhibition of PASMC \(K_v\) channels would be seen. In the normotensive pulmonary circulation ET\(_A\) blockade alone would not be sufficient to prevent ET-1-mediated inhibition of \(I_{K(v)}\). However, our data are consistent with the hypothesis that the high degree of efficacy of ET\(_A\)-receptor blockade in vivo could be explained by the switch in coupling between ET-1 and \(K_v\) channels from ET\(_B\) to ET\(_A\) receptors.

In conclusion, we found that ET-1 inhibited \(K_v\) channels in PASMC from both normoxic and chronically hypoxic rats. Although we did not directly measure the change in membrane potential caused by inhibition of \(I_{K(v)}\), the dependence of PASMC on \(I_{K(v)}\) to set resting membrane potential suggests that ET-1 could cause membrane depolarization through this mechanism. Chronic hypoxia resulted in a switch in receptor coupling, eliminating the absolute requirement for ET\(_B\) activation seen in normoxic PASMC, although the maximum effect was seen when both ET\(_A\) and ET\(_B\) receptors were inhibited. Therefore, our findings are consistent with the hypothesis that at least a portion of the activity of ET-1 in the hypoxic pulmonary circulation is inhibition of \(K_v\) channels mediated by activation of ET\(_A\) and, to a lesser extent, ET\(_B\) receptors. This effect, exerted on a background of increased ET-1 production and constitutively depolarized PASMC, would prime the PASMC for further hypoxic inhibition of \(K_v\) channels resulting in membrane depolarization, activation of voltage-gated \(Ca^{2+}\) channels, and, ultimately, pulmonary artery vasoconstriction.

This work was supported by National Heart, Lung, and Blood Institute Grants HL-48038 (D. M. Rodman) and HL-14985 (D. M. Rodman, I. F. McMurtry).

Address for reprint requests and other correspondence: D. M. Rodman, Campus Box B-133, Univ. of Colorado Health Sci. Ctr., 4200 E. Ninth Ave., Denver, Colorado 80262 (E-mail: David.Rodman@uchsc.edu).

Received 7 October 1998; accepted in final form 10 February 1999.

REFERENCES


7. Elton, T. S., S. Oparil, G. R. Taylor, P. H. Hicks, R. H. Yang, H. J. in, and Y. F. Chen. Normobaric hypoxia stimulates endothelin-
19. Muramatsu, M. D. M. Rodman, M. Oka, and I. V. McMurtry. Endothelin-1 mediates nitro-l-arginine vasconstriction of hyper-
23. Sailer, K. J. , and R. Z. Kozlowski. Differential electrophysiologi-
28. Tohse, N., and N. Sperelakis. 8-Br-cyclic GMP inhibits the calcium channel current in embryonic chick ventricular myo-
30. Weir, E. K., H. L. Reeve, D. N. Cornfield, M. Tristani-
31. Wu, S.-N., H.-S. Yu, and Y. Seyama. Induction of Ca2+ oscilla-