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Effects of Hypoxia on Pulmonary Vascular Contractility

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Although hypoxic pulmonary vasoconstriction (HPV) has been recognized by many researchers, the precise mechanism remains unknown. As isolated pulmonary arteries will constrict in vitro in response to hypoxia, the oxygen sensor/transduction mechanism must reside in the pulmonary arterial smooth muscle or in the endothelium, or in both. Unfortunately, much of the current evidence is conflicting, especially as to the dependency of HPV on the endothelium and the role of a K^+ channel. Therefore, this experiment was attempted to clarify the dependency of HPV on the endothelium and the role of a K^+ channel on HPV in rat pulmonary artery. The effects of hypoxia were investigated in isolated main pulmonary arteries precontracted with norepinephrine. Vascular rings were suspended for isometric tension recording in an organ chamber filled with a Krebs-Henseleit solution. Hypoxia was induced by gassing the chamber with 95% N_2 + 5% CO_2 and this was maintained for 20 min. Hypoxia elicited a vasoconstriction in arteries with endothelium. Mechanical disruption of the endothelium abolished HPV. There was no difference between the amplitude of the HPV induced by two consecutive hypoxic challenges and the effect of normoxic and hyperoxic control Krebs-Henseleit solution on a subsequent response to hypoxia. Inhibition of NO synthesis by treatment with N^{ω}-nitro-L-arginine reduced HPV, but inhibition of a cyclooxygenase pathway by treatment with indomethacin had no effect on HPV. Blockades of a tetraetylammonium chloride-sensitive K^+ channel abolished HPV. Verapamil, a Ca^{2+} entry blocker reduced HPV. In conclusion, these results suggest that HPV was dependent on the endothelium and that HPV can be considered to be induced by inhibition of the mechanisms of NO-dependent vasodilation such as the opening of a K^+ channels.

Key Words: Hypoxic pulmonary vasoconstriction, nitric oxide, K^+ channel

Hypoxic pulmonary vasoconstriction (HPV) is a well-known adaptive mechanism for the matching of regional blood flow to regional ventilation in the lung (Demiryurek *et al.* 1991).

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Although HPV has been recognized by many researchers (Rodman *et al.* 1990; Yuan *et al.* 1990; Bennie *et al.* 1991; Robertson *et al.* 1992), the precise mechanism remains unknown. As isolated pulmonary arteries will constrict in vitro in response to hypoxia, the oxygen sensor/transduction mechanism must reside in the pulmonary arterial smooth muscle, in the endothelium, or in both. Unfortunately, much of the current evidence is conflicting, especially as to the dependency of HPV on the endothelium and the role of a K^+ channel. There have been reports of complete (Graser and Vanhoutte, 1991; Muramatsu *et al.* 1992) and partial endothelium dependency (Johns *et al.* 1989) as well as endothelium independency (Ohe *et al.* 1992) of

HPV in isolated pulmonary arteries.

The first aim of this study was to determine the effects of hypoxia on isometric tension development in norepinephrine (NE)-precontracted rat pulmonary artery rings. The second aim was to elucidate the role of the endothelium in HPV. The third aim was to elucidate the effects of cyclooxygenase inhibition, as well as K^+ channel and Ca^{2+} channel blockade on the HPV.

MATERIALS AND METHODS

Preparation of artery rings and conditions of hypoxia

Rats were killed by cervical dislocation. The chest was opened and the heart and lungs were placed in preoxygenated Krebs-Henseleit (KH) solution (mM: NaCl 119, KCl 4.6, $CaCl_2$, 2.5, $NaHCO_3$ 25, $MgCl_2$ 1.2, KH_2PO_4 1.2, glucose 11) at room temperature. The pulmonary artery was carefully dissected to avoid stretching and then removed. The main pulmonary artery was then carefully dissected free of fat and extraneous tissue and cut into 3mm rings. In some rings, the endothelium was removed by rotating the ring gently on the tip of a forceps. The rings were then mounted in water-jacketed baths containing KH solution at 37°C, gassed with 95% O_2 + 5% CO_2 (hyperoxic gas) or 20% O_2 + 5% CO_2 + 75% N_2 (normoxic gas) and connected to force transducers to measure isometric tension. A resting tension of 2*g* was maintained throughout the experiments. Tissues were allowed to equilibrate for 90min before each experiment. The function of the endothelium was checked at the beginning of each experiment with acetylcholine ($5X10^{-7}$M). Hypoxia of norepinephrine (NE)-precontracted rings was induced by bubbling with 95% N_2 + 5% CO_2 gas instead of hyperoxic or normoxic gas for 20min. The P_{O2} of the KH solution in the tissue baths was determined with a blood gas analyzer (Radiometer, Westlake, Ohio U.S.A.) during each cycle of each experiment. The mean dissolved partial pressures of oxygen in the bath fluid during hyperoxia and normoxia cycles were 543±8 mmHg and 134±12 mmHg, respectively and during hypoxia, 30.1±0.3 mmHg.

Influence of drugs on hypoxic responsiveness

Resting state (absence of vasoconstrictor agonist) rings were exposed to hypoxia for 20 min. After 45 min of recovery under hyperoxic gas, the rings were contracted with NE (10^{-7}M) and exposed again to hypoxia. After an additional period of 15 min of hyperoxic gas, the following inhibitor and/or blocker were administered for 30 min: N^{ω}-nitro-L-arginine (L-NNA) [a blocker of a nitric oxide synthesis] (Palmer and Moncada, 1989), indomethacin [an inhibitor of cyclooxygenase pathway (Miller and Vanhoutte, 1985)], tetraethylammonium chloride [TEA; a blocker of a non-specific K^+ channel (Post *et al.* 1992)], verapamil [a blocker of a voltage-dependent Ca^{2+} channel (Archer *et al.* 1985)]. In the presence of these drugs, the effects of hypoxia were tested under precontracted arteries.

Data analysis

Results were expressed as the mean±SE. The number of preparations taken from separate animals was indicated by n. The tension development by hypoxia in NE-precontracted ring was expressed as percent peak amplitude of 40 mM K^+-induced contraction. Significance tests were performed by Student's paired or unpaired t-test. P values of less than 0.05 were considered significant.

RESULTS

Effects of hypoxia on precontracted arteries with and without endothelium

In endothelium-containing rings of rat pulmonary artery partially contracted with NE (10^{-7}M), changing the aerating gas from 95% O_2 + 5% CO_2 to 95% N_2 + 5% CO_2 produced a contraction (Fig. $1A_1$). The peak contraction was attained 5 min later, and the tension was maintained until reoxygenation. Removal of endothelium eliminated the contractile effect of hypoxia and produced a small relaxation in some preparations (Fig. $1A_2$). The amplitude of hypoxia-induced contraction in NE-precontracted artery with and without endothelium was 122.4± 13% (n=30, $P<0.05$) and 11.5±3% (n=11), respec-

tively (Fig. 1B).

In NE-precontracted artery with endothelium, hypoxia-induced contraction did not differ significantly between the two consecutive hypoxic challenges (Fig. 2). The amplitude of hypoxia-induced contraction was 130.9±16.2% (1st episode; n=10) and 132.3±21.9% (2nd episode; n=10), respectively. Therefore, the hypoxia-induced contraction was reproducible. As shown in Fig. 3, the amplitude of hypoxia-induced contraction did not differ between arteries equilibrated with normoxic gas and hyperoxic gas. The mean amplitude of hypoxia-induced contraction was 128.4±13.5% (normoxic gas; n=11) and 153.0±39.4% (hyperoxic gas; n=11).

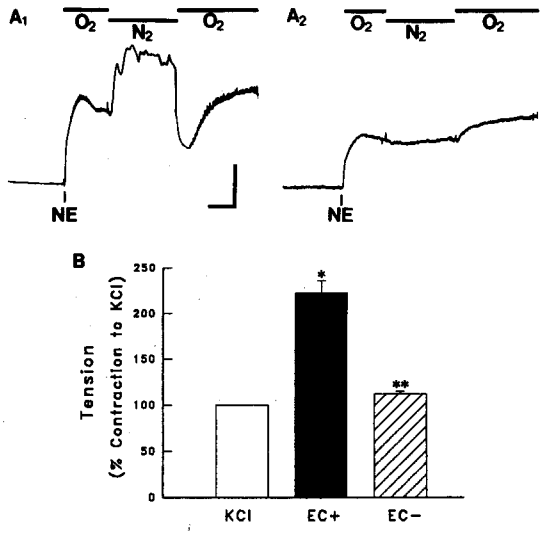

*Fig. 1. Effect of hypoxia on contractile responses in rat pulmonary arteries. A_1, A_2: shows typical response to hypoxia in rings of pulmonary artery with (A_1) and without (A_2) endothelium. B: shows mean response of pulmonary artery with (EC+; n=30) and without (EC-; n=11) endothelium under the same conditions. The preparations were contracted with norepinephrine (NE; 10^{-7} M). Hypoxia was induced by switching from 95% O_2 + 5% CO_2 (O_2) to a 95% N_2±5% CO_2 gas mixture (N_2). Data are expressed as mean±SE. *significant difference between 40 mM K^+-induced contraction and hypoxia-induced contraction ($P<0.05$). **significant difference between preparations with and without endothelium ($P<0.05$). Horizontal scale bar: 10 min, Vertical scale bar: 100 mg.*

Effects of L-NNA and indomethacin on hypoxia-induced contraction

The effects of nitric oxide synthase inhibitor was tested by adding 10^{-3}M N^{ω}-nitro-L-arginine (L-NNA) to one each of nine paired rings (Fig. 4). In resting pulmonary artery rings, L-NNA increased resting

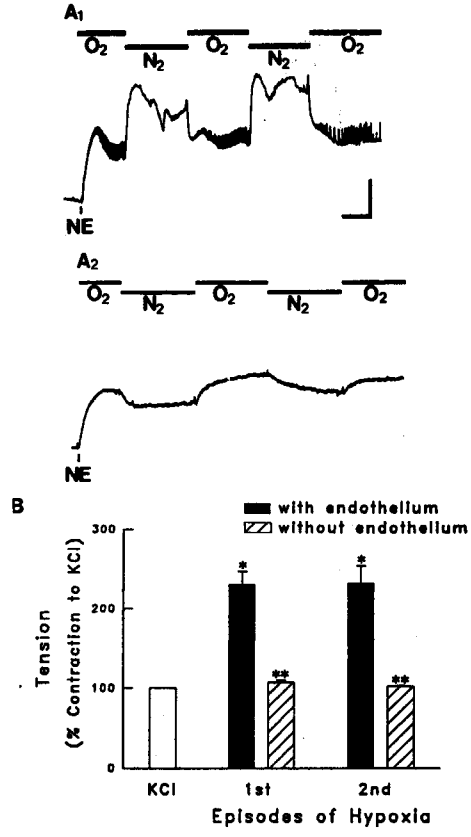

*Fig. 2. Reproducibility of two consecutive hypoxic challenges to pulmonary arteries. A_1, A_2: shows typical response to two consecutive hypoxic challenges in precontracted (norepinephrine; NE, 10^{-7} M) pulmonary artery with (A_1) and without (A_2) endothelium. B: shows mean response of pulmonary artery with (EC+; n=10) and without (EC-; n=7) endothelium under the same conditions. Data are expressed as mean±SE. *significant difference between 40 mM K^+-induced contraction and hypoxia-induced contraction ($P<0.05$). **significant difference between preparations with and without endothelium ($P<0.05$). Horizontal scale bar: 10 min, Vertical scale bar: 100 mg. Solid bar with endothelium. Hatched bar without endothelium.*

force. Treatment with L-NNA markedly inhibited the contraction caused by hypoxia in NE-precontracted pulmonary arteries (control group; 150.5±30.6%, L-NNA treated group; 11.3±5.6%, $P<0.05$).

The effects of cyclooxygenase inhibition on the hypoxia-induced contraction was tested by adding 10^{-5}M indomethacin (Fig. 5). Treatment with indomethacin had no effect on hypoxia-induced contraction (control group; 128.4±38.5%, indomethacin treated group; 195.6±69.2%, n=8).

Effects of TEA and verapamil on hypoxia-induced contraction

In NE-precontracted arteries, 1mM tetraethylammonium chloride (TEA), a non-specific K^+ channel blocker, appeared to augment the NE-induced tone about 91.5±8.4% (n=8), whereas TEA blocked the hypoxia-induced contraction (Fig. 6). The amplitudes of hypoxia-induced contraction with and without TEA were -3.1±4.7% and 101.5±12.4%, respectively.

In six pulmonary artery rings precontracted with NE, 10^{-5}M verapamil, a blocker of a voltage-dependent Ca^{2+} channel, reduced hypoxia-induced contraction (Fig. 7). The amplitudes of hypoxia-induced contraction with and without verapamil were 14.1±5.7% and 128.5±10.4%, respectively.

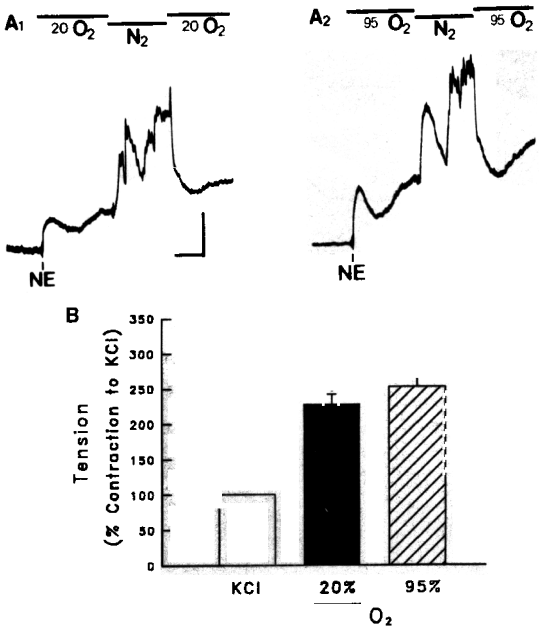

*Fig. 3. Effect of normoxic or hyperoxic control Krebs-Henseleit solution on subsequent response to hypoxia in pulmonary arteries with endothelium. A_1, A_2: shows typical recording to effect of normoxic (A_1) or hyperoxic (A_2) control Krebs-Henseleit solution on subsenquent response to hypoxia in precontracted (norepinephrine; NE, 10^{-7} M) pulmonary artery. B : shows mean response of pulmonary artery incubated with normoxic (20% O_2) and hyperoxic (95% O_2) control Krebs-Henseleit solution under the same conditions (n=11). Data are expressed as mean±SE. *significant difference between 40 mM K^+-induced contraction and hypoxia-induced contraction ($P<0.05$). Horizontal scale bar: 10 min, Vertical scale bar: 100 mg.*

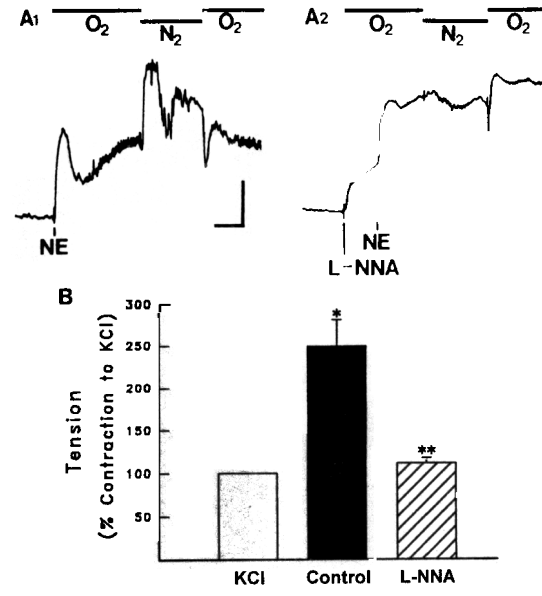

*Fig. 4. Effect of inhibiting NO synthesis by N^{ω}-nitro-L-arginine on response to hypoxia in pulmonary arteries with endothelium. A_1, A_2: shows typical response to hypoxia in precontracted (norepinephrine; NE, 10^{-7} M) pulmonary artery with (A_2) and without (A_1) N^{ω}-nitro-L-arginine (L-NNA, 10^{-5} M). B: shows mean response of pulmonary artery with (L-NNA) and without (Control) N^{ω}-nitro-L-arginine under the same conditions (n=9). N^{ω}-nitro-L-arginine was applied 30~40 min before testing their efficacy. Data are expressed as mean±SE. *significant difference between 40 mM K^+-induced contraction and hypoxia-induced contraction ($P<0.05$). **significant difference between preparations with and without N^{ω}-nitro-L-arginine ($P<0.05$). Horizontal scale bar: 10 min, Vertical scale bar: 100 mg.*

DISCUSSION

Previous investigations of HPV in isolated pulmonary arteries have resulted in some apparently inconsistent hypotheses (Yuan *et al.* 1990). Their study demonstrated that hypoxia produces a contraction of rat isolated pulmonary artery rings both under resting tension (data not shown) and when precontracted with NE. The HPV in precontracted arteries was larger than that observed under resting tension, showing that precontraction is not essential but amplifies the HPV. In rat pulmonary arteries, HPV has been observed in the presence of angiotensin, phenylephrine, U46619 and KCl (Rodman *et al.* 1989) implying that the hypoxic response is not dependent on a specific agonist-receptor interaction. Other evidence from this study suggests that HPV in precontracted arteries with endothelium is reproducible and the brief episodes of hypoxia do not impair the response of endothelium.

One of the major findings of this study is that in endothelium-denuded arteries, HPV was abolished, and in some preparations a small relaxation occurred in NE-precontracted arteries. These results suggest that HPV is endothelium-dependent and the HPV results from changes in release, caused by hypoxia, of mediators from the endothelium; there could be an increased release of a contractile element such as

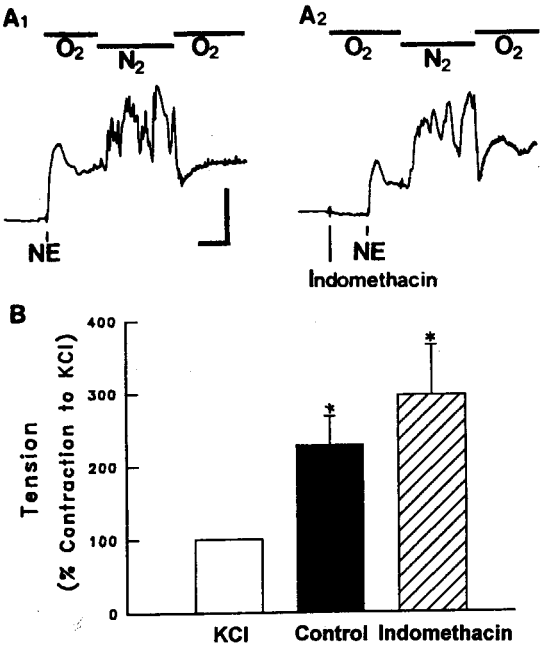

*Fig. 5. Effect of blockading cyclooxygenase pathways by indomethacin on response to hypoxia in pulmonary arteries with endothelium. A_1, A_2: shows typical response to hypoxia in precontracted (norepinephrine; NE, 10^{-7} M) pulmonary artery with (A_2) and without (A_1) indomethacin (10^{-5} M). B: shows mean response of pulmonary artery with (Indomethacin) and without (Control) indomethacin under the same conditions (n=8). Indomethacin was applied 30~40 min before testing their efficacy. Data are expressed as mean±SE. *significant difference between 40 mM K^+-induced contraction and hypoxia-induced contraction ($P<0.05$). Horizontal scale bar: 10 min, Vertical scale bar: 100 mg.*

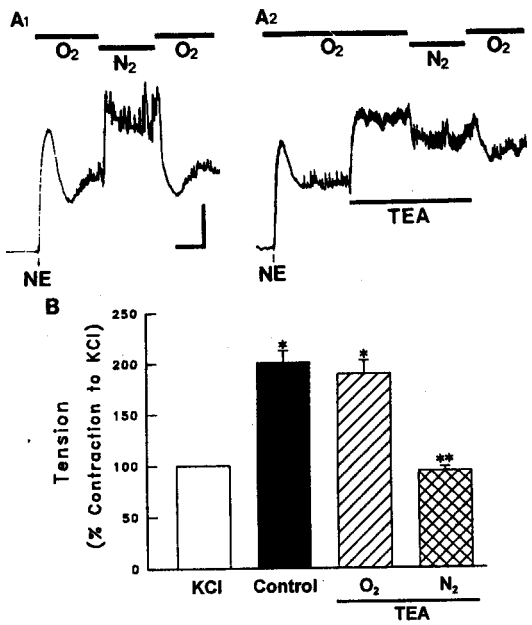

*Fig. 6. Effect of TEA on response to hypoxia in pulmonary arteries with enodthelium. A_1, A_2: shows typical response to hypoxia in precontracted (norepinephrine; NE, 10^{-7} M) pulmonary artery with (A_2) and without (A_1) TEA (1 mM). B : shows mean response of pulmonary artery with (TEA) and without (Control) TEA under the same conditions (n=8). TEA was applied after norepinephrine-induced precontraction. Data are expressed as mean±SE. *significant difference between 40 mM K^+-induced contraction and hypoxia- or TEA-induced contraction ($P<0.05$). Horizontal scale bar: 10 min, Vertical scale bar: 100 mg.*

endothelium-derived contracting factor (Green and Leffler, 1984; Madden *et al.* 1986; Rabinovitch *et al.* 1989) and/or reduced release of a vasodilator mediator such as nitric oxide (Holden and McCall, 1984; Robertson *et al.* 1990).

In this study L-NNA caused a contraction in resting arteries and inhibited the contractile effects of hypoxia in precontracted arteries, but indomethacin has no effect on the HPV in precontracted arteries. L-NNA is a blocker of nitric oxide synthesis (Palmer and Moncada, 1989) and indomethacin is an inhibitor of the cyclooxygenase pathway (Miller and Vanhoutte, 1985). These results suggest that HPV may be caused by inhibition of the basal release of nitric oxide not related to cyclooxygenase.

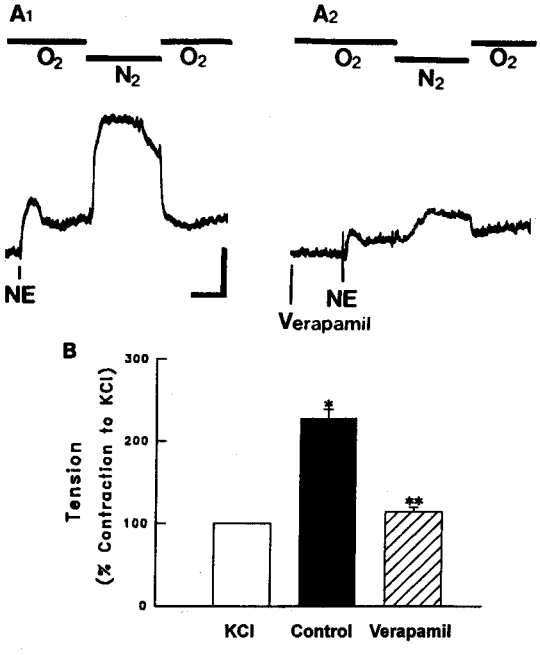

*Fig. 7. Effect of verapamil on response to hypoxia in pulmonary arteries with endothelium. A_1, A_2: shows typical response to hypoxia in precontracted (norepinephrine; NE, 10^{-7} M) pulmonary artery with (A_2) and without (A_1) verapamil (10^{-5} M). B: shows mean response of pulmonary artery with (Verapamil) and without (Control) verapamil under the same conditions (n=6). Verapamil was applied 20 min before testing their efficacy. Data are expressed as mean ±SE. *significant difference between 40 mM K^+-induced contraction and hypoxia-induced contraction ($P<0.05$). Horizontal scale bar: 10 min, Vertical scale bar: 100 mg.*

If the endothelium was continuously releasing inhibitory substances such as nitric oxide, which in turn inhibit contraction of the smooth muscle due to NE (Vanhoutte, 1987), hypoxia could cause contraction by preventing this release. However, it has been demonstrated that following administration of L-NNA, the response to hypoxia is significantly increased (Feng *et al.* 1994). The differences between this study and opposing results remains uncertain.

It has been demonstrated that hypoxia causes depolarization of the resting membrane potential in intact pulmonary arterial smooth muscle (Bradford and Dean, 1994) and subsequent Ca^{2+} entry through a voltage dependent Ca^{2+} channel (Cornfield *et al.* 1994). Bay K 8644, a Ca^{2+} channel agonist, augments HPV, whereas dihydropyridine Ca^{2+} channel blockers inhibit HPV (Cornfield *et al.* 1994). In this study, TEA, a blocker of a non-specific K^+ channel (Post *et al.* 1992) and verapamil, a blocker of voltage-dependent Ca^{2+} channels (Archer *et al.* 1985), both inhibited HPV in precontracted arteries. These results suggest that hypoxia may inhibit K^+ channels and produce membrane depolarization, leading to an increase in Ca^{2+} entry through voltage-dependent Ca^{2+} channels. It has been demonstrated that TEA and 4-aminopyridine causes vasoconstriction in the normoxic lung and that nifedipine inhibits HPV (Hasunuma *et al.* 1991). These results suggested that Ca^{2+} and/or voltage-dependent K^+ channels may be important in mediating pulmonary vascular tone. But HPV was dependent on endothelium in this study. Therefore, the ability of these agents to mimic the effects of hypoxia may not be direct effects on pulmonary artery smooth muscle.

In conclusion, this study suggests that HPV was dependent on the endothelium and was considered to be induced by inhibition of the mechanism of nitric oxide-dependent vasodilation such as the opening of K^+ channels.

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