# Hypoxia Inhibits Gene Expression of Voltage-gated $K^+$ Channel $\alpha$ Subunits in Pulmonary Artery Smooth Muscle Cells

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## Abstract

**Activity of voltage-gated $K^+$ channels ($K_V$) in pulmonary arterial smooth muscle cells (PASMC) is pivotal in controlling membrane potential, cytoplasmic free $Ca^{2+}$ concentration ($[Ca^{2+}]_{cyt}$), and pulmonary vasomotor tone. Acute hypoxia selectively inhibits $K_V$ channels, depolarizes PASMC, raises $[Ca^{2+}]_{cyt}$, and causes pulmonary vasoconstriction and vascular remodeling. Prolonged hypoxia (24–60 h) decreased significantly the mRNA levels of $K_V$ channel $\alpha$ subunits, $K_V1.2$ and $K_V1.5$. Consistently, the protein levels of $K_V1.2$ and $K_V1.5$ were also decreased significantly by hypoxia (48–72 h). Nevertheless, hypoxia affected negligibly the mRNA levels of $K_V$ channel $\beta$ subunits ($K_V\beta1$, $K_V\beta2$, and $K_V\beta3$). The native $K^+$ channels are composed of pore-forming $\alpha$ and auxiliary $\beta$ subunits. Assembly of $K_V$ $\beta$ subunits with $\alpha$ subunits confers rapid inactivation on the slowly or non-inactivating delayed rectifier $K_V$ channels. $K_V$ $\beta$ subunits also function as an open-channel blocker of $K_V$ channels. Thus, the diminished transcription and expression of $K_V$ $\alpha$ subunits may reduce the number of $K_V$ channels and decrease $K_V$ currents. Unchanged transcription of $K_V$ $\beta$ subunits may increase the fraction of the $K_V$ channel $\alpha$ subunits that are associated with $\beta$ subunits and further reduce the total $K_V$ currents. These data demonstrate a novel mechanism by which chronic hypoxia may cause pulmonary vasoconstriction and hypertension. (*J. Clin. Invest.* 1997. 100: 2347–2353.) Key words: $K_V1.2$ • $K_V1.5$ • $\alpha$ subunits • $\beta$ subunits • reverse transcription-PCR • Western blotting**

## Introduction

In pulmonary arterial smooth muscle cells (PASMC),[1] activity of voltage-gated $K^+$ ($K_V$) channels is an important determinant in controlling resting membrane potential ($E_m$) (1–3) which, in turn, regulates cytosolic free calcium concentration ($[Ca^{2+}]_{cyt}$) because of the voltage dependence of sarcolemmal $Ca^{2+}$ channels (1, 4). Elevation of $[Ca^{2+}]_{cyt}$ in PASMC is a major trigger for pulmonary vasoconstriction (5) and vascular smooth muscle cell proliferation (leading to vascular remodeling) (6, 7).

Acute hypoxia (< 3 min) inhibits $K_V$ channels in PASMC (3, 8–10). The resultant decrease in $K_V$ currents ($I_{K(V)}$) depolarizes the myocytes, increases $[Ca^{2+}]_{cyt}$, and causes pulmonary vasoconstriction (11–14). Chronic exposure to hypoxia (1–20 d) causes a steady increase in pulmonary arterial pressure that is significant by day 2 and maximized by day 20 (15). An early work by McMurtry et al. (16) indicates that the pressor response to acute hypoxia in lungs from chronically hypoxic rats is decreased significantly, while the response to vasoconstrictor agonists (angiotensin II, prostaglandin $F_{2\alpha}$, and norepinephrine) is augmented. They suggest that this reduced pressor responsiveness may result from abnormalities in the mechanism that couples acute hypoxia with contraction of the pulmonary vascular smooth muscle (16). Recently, reduced $I_{K(V)}$ and associated membrane depolarization have been observed in PASMC isolated from chronically hypoxic rats (17, 18). These data imply that chronic hypoxia may interact directly with the coupling mechanism (e.g., the $K_V$ channels) by which acute hypoxia causes pulmonary vasoconstriction.

$K_V1.2$ and $K_V1.5$ are two *Shaker*-like $K_V$ channel $\alpha$ subunits cloned recently from smooth muscle cells (19, 20). Adda et al. (21) identified $K_V1.2$ and $K_V1.5$ in human airway smooth muscle cells. In rat PASMC, we have found recently that, in addition to expressing $K_V1.2$ and $K_V1.5$, the cells also express three $K_V$ channel $\beta$ subunits ($K_V\beta1$, $K_V\beta2$, and $K_V\beta3$) (22). Electrophysiological studies on the expressed $K_V1.2$ and $K_V1.5$ channels indicate that these channels are slowly or non-inactivating delayed rectifier $K_V$ channels and are sensitive to the $K_V$ channel blocker, 4-aminopyridine (19, 20, 23, 24). Activity of the 4-aminopyridine–sensitive $K_V$ channels in PASMC plays a critical role in regulating $E_m$ and $[Ca^{2+}]_{cyt}$ (1–4) and in initiating hypoxia-mediated membrane depolarization and vasoconstriction (3, 8–10, 17).

$K_V$ $\beta$ subunits can bind specifically to the *Shaker*-like $K_V$ $\alpha$ subunits ($K_V1$ subfamily) (25, 26) through a highly conserved region in the amino-terminal domains (amino-terminal A and B box) of $\alpha$ subunits (27). Association of $K_V$ channel $\beta$ subunits with $K_V1.2$ or $K_V1.5$ alters profoundly the biophysical properties of the channels (26, 28, 29). Coexpression of $K_V\beta1$ with $K_V1.2$ or $K_V1.5$ not only confers rapid inactivation on these slowly or non-inactivating delayed rectifier channels (26,

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1. *Abbreviations used in this paper:* $[Ca^{2+}]_{cyt}$, cytoplasmic free calcium concentration; $E_m$, resting membrane potential; eNOS, nitric oxide synthase; HIF-1, hypoxia-inducible factor 1; $I_K$, total potassium currents; $K_V$, voltage-gated $K^+$ channels; PASMC, pulmonary arterial smooth muscle cells; $PO_2$, oxygen tension; RT-PCR, reverse transcription-PCR.

28, 29), but also inhibits the activity of $K_V$ channels as an open-channel blocker (30).

In this study, the effects of prolonged hypoxia (1–3 d) on the mRNA and protein levels of $K_V$ channel α subunits ($K_V$1.2 and $K_V$1.5) and β subunits ($K_V$β1, $K_V$β2, and $K_V$β3) were determined to test the hypothesis that transcriptional regulation of $K^+$ channels by chronic hypoxia plays an important role in the development of pulmonary hypertension.

## Methods

*Cell culture and treatment with hypoxia.* Primary cultured PASMC were obtained from rat intrapulmonary arteries (third or fourth division) and branches of the main pulmonary artery (second division). The methods used to dissociate the cells and to prepare the cultures were described previously (1). The cells, grown on 10-cm petri dishes, were fed twice a week with 10% fetal bovine DME (containing 5.5 mM glucose) and incubated in a humidified atmosphere containing 5% $CO_2$ in air at 37°C for 5–7 d before experiments. The cells were then divided into six groups. Group 1 was incubated continuously in the incubator containing 5% $CO_2$ in air (normoxia; the oxygen tension [$P_{O_2}$], was 130–140 Torr). The other groups of cells (groups 2–6) were placed in an $O_2$-regulated incubator (Forma Scientific, Inc., Marietta, OH) at 3% $O_2$/5% $CO_2$/92% $N_2$ (hypoxia; $P_{O_2}$ was 25–35 Torr) for 24, 36, 48, 60, and 72 h, respectively. The time of hypoxia (24–72 h) used in this study was selected because 48 h of hypoxia increases significantly pulmonary arterial pressure (15). $P_{O_2}$ in the cell culture media reaches ambient $P_{O_2}$ within 2 h after placement of the petri dishes in the hypoxic incubator. There were no significant changes in pH values (by 0.07±0.02) of the culture media or in cell viability under normoxic or hypoxic (24–72 h) conditions.

*Reverse transcription-PCR (RT-PCR).* Total RNA was prepared from the primary cultured PASMC by the acid guanidinium thiocyanate-phenol-chloroform extraction method (31). Isolated total RNA was dissolved in dietryl pyrocarbonate water at 1 μg/μl, and stored at −70°C. Reverse transcription (RT) was performed using the First-Strand cDNA Synthesis kit (Pharmacia Biotech, Piscataway, NJ). 3 μg of the total RNA was reverse-transcribed using random hexamers [pd(N)$_6$ primer]. The reaction mixture was incubated for 1 h at 37°C and then heated at 90°C for 5 min to inactivate the reverse transcriptase.

Specific primers for $K_V$ channel α and β subunits were designed from the cDNA sequences of the coding regions corresponding to rat $K^+$ channel genes. Primers for $K_V$1.2 (295 bp) and $K_V$1.5 (1,111 bp) were designed from coding regions of BK2 (GenBank accession no. J04731) and KV1 (M27158), respectively. Primers for $K_V$ channel β subunits were designed from $K_V$β1 (X70662, 150 bp), $K_V$β2 (X76724, 141 bp), and $K_V$β3 (X76723, 178 bp), respectively (see Table I).

PCR was performed by the GeneAmp PCR system (model 2400; Perkin-Elmer Corp., Norwalk, CT) using *Taq* polymerase and accompanying buffers. 3 μl of the first strand cDNA reaction mixture was used. The cDNA samples were amplified in the Perkin-Elmer DNA thermal cycler under the following conditions: the mixture was annealed at 55°C (1 min), extended at 72°C (2 min), and denatured at 94°C (1 min) for 25 cycles (Table I). This was followed by a final extension at 72°C (10 min) to ensure complete product extension. The PCR products were electrophoresed through a 1% agarose gel, and amplified cDNA bands were visualized by ethidium bromide staining. Since β-actin mRNA levels were much higher than $K_V$ channel mRNA levels, only half the amount of the β-actin PCR products (5 μl) relative to $K_V$ channel products (10 μl) was used for electrophoresis.

To quantify the PCR products (the amounts of mRNA) of $K_V$ channels (α and β subunits), an invariant mRNA of β-actin was used as an internal control. Immediately after each experiment, the OD values for each band on the gel were measured by the Gel Documentation system (UVP Inc., Upland, CA). The OD values in $K^+$ channel signals were normalized to the OD values in the β-actin signals. The normalized values in the normoxic controls were expressed as 1 arbitrary U for quantitative comparison. Since PCR amplification is an exponential process, the extent of amplification is not only dependent on the initial amount of target mRNA (or cDNA), but also related to efficiency and cycle number. Although the invariant β-actin mRNA was used as internal control, the possible difference in efficiency between the primer pairs for β-actin and the target mRNA can still lead to different yield of PCR products. Therefore, the PCR study provides only a relative comparison of amounts of mRNA.

*Immunoblotting.* The primary cultured PASMC were washed with PBS, scraped into PBS (2 ml/dish), and centrifuged at 3,500 rpm. The cell pellet was homogenized in the EXTRA buffer (1% deoxycholic acid, 1% Igepal CA-630, 10 mM $NaH_2PO_4$, 140 mM NaCl, 2 mM EDTA, and 10 mM sodium azide) containing the protease inhibitor cocktail (Complete® tablets; Boehringer Mannheim Biochemicals, Indianapolis, IN) with a Polytron homogenizer (Brinkmann Instruments, Inc., Westbury, NY) for 10 s at 7,000 rpm. Protein concentrations were determined by the BCA protein assay (Pierce Chemical Co., Rockford, IL), using BSA as a standard. The samples of homogenates were used for immunoblotting.

*Table I. Characteristics of Primers and Conditions of RT-PCR*

| Name | Primer | Sequence | Location | Fragment size | Number of cycles | Total [RNA] | [cDNA] |
|---|---|---|---|---|---|---|---|
| | | | *nt* | *bp* | | *μg* | *μl* |
| $K_v$1.2 | Sense | 5′-TACATGGAGATACAGGAGG-3′ | 1953–1971 | 295 | 25 | 3 | 3 |
| (J04731)* | Antisense | 5′-ATATTCTGTGTTCTAAATCA-3′ | 2228–2247 | | | | |
| $K_v$1.5 | Sense | 5′-GCCTGGAGACTCTGCCTGAGTTCAGGGATG-3′ | 1536–1565 | 1111 | 25 | 3 | 3 |
| (M27158)* | Antisense | 5′-GGTGTAAAGCAGATGCCCAGGCTCAAGGGG-3′ | 2617–2646 | | | | |
| $K_v$β1 | Sense | 5′-AGGACTATAGATCCTAAGGC-3′ | 1521–1540 | 150 | 25 | 3 | 3 |
| (X70662)* | Antisense | 5′-CTCAGAGAATCCTGGGACAC-3′ | 1651–1670 | | | | |
| $K_v$β2 | Sense | 5′-ATAGCCTGGTGCCTGAGGAA-3′ | 1514–1533 | 141 | 25 | 3 | 3 |
| (X76724)* | Antisense | 5′-AATGCTGTCGATCTCGTGGA-3′ | 1635–1654 | | | | |
| $K_v$β3 | Sense | 5′GAGTGATTGCACCCTTTGGA-3′ | 1631–1650 | 178 | 25 | 3 | 3 |
| (X76723)* | Antisense | 5′-CACGGTGAAAGGATATGGCT-3′ | 1789–1808 | | | | |
| β-Actin | Sense | 5′-AGTGTGACGTTGACATCCGT-3′ | 2731–2750 | 244 | 25 | 3 | 3 |
| (J00691)* | Antisense | 5′-GACTCATCGTACTCCTGCTT-3′ | 3079–3098 | | | | |

*GenBank accession numbers for the sequences used in designing the primers.

Proteins solubilized in SDS buffer were separated by SDS-PAGE. The 10% gels were calibrated with prestained protein molecular weight markers (Bio-Rad Laboratories, Richmond, CA). Proteins were then transferred to the Hybond-C extra nitrocellulose membrane (Amersham Corp., Arlington Heights, IL) as described (32). The efficiency of the transfer was verified by Ponceau-S staining. Membranes were blocked with 5% nonfat dry milk in Tris-buffered saline and 0.1% Tween 20. The blots were then incubated with the affinity-purified polyclonal antibodies specific for $K_V1.2$ (1:300, Alomone Labs, Jerusalem, Israel), $K_V1.5$ (1:1,000; Upstate Biotechnology Inc., Lake Placid, NY), and α-actin (1:1,000; Boehringer Mannheim Biochemicals). The membranes were washed three times for 5 min each and incubated with anti–rabbit or anti–mouse horseradish peroxidase–conjugated IgG for 1 h, and an enhanced chemiluminescence detection system (ECL; Amersham Corp.) was used for detection of the bound antibody.

*Statistical analysis.* The composite data are expressed as means±SE. Statistical analyses were performed using paired Student's *t* test. Differences were considered to be significant when $P < 0.05$.

## Results

The quantity of PCR products for β-actin and $K_V1.2$ correlated linearly with the change of cycle numbers between 23 and 28 cycles, while 3.0 μg total RNA and 3.0 μl cDNA were used in RT-PCR (Fig. 1 *A*). With 25 cycles and 3.0 μg total RNA used for amplifying the messages in PCR, the change in cDNA level of β-actin and $K_V\beta3$ between 1.5 and 5.0 μl correlated linearly with the amount of the PCR products (Fig. 1 *B*). When 3.0 μl cDNA and 25 cycles were used in PCR, the change in total RNA levels between 1.5 and 5.0 μg also correlated linearly with the quantity of the PCR products of β-actin, $K_V1.2$, and $K_V\beta3$ (Fig. 1 *C*). These results indicate that the experimental protocol for RT-PCR used in this study (3 μg total RNA for RT, 3 μl cDNA and 25 cycles for PCR) was appropriate to quantify the mRNA levels of $K_V$ channels.

*Effects of hypoxia on mRNA levels of $K_V$ channel α subunits.* Total RNA was extracted from primary cultured rat PASMC incubated under normoxic (5% $CO_2$ in air, $Po_2$ = 130–140 Torr) and hypoxic (3% $O_2$/5% $CO_2$ in $N_2$, $Po_2$ = 25–35 Torr) conditions, respectively. After RT, the same amount of first-strand cDNA from each of the normoxic and hypoxic cells was used in PCR consisting of the specific primers for $K^+$ channels and β-actin. The gene transcription (mRNA levels) of $K_V$ channel α subunits ($K_V1.2$ and $K_V1.5$) and β subunits ($K_V\beta1$, $K_V\beta2$ and $K_V\beta3$) were examined, and the β-actin mRNA level was used as control.

The mRNA levels of $K_V1.2$ and $K_V1.5$ were decreased significantly by exposure to hypoxia in a time-dependent manner (Fig. 2, *A* and *B*). The inhibition of $K_V1.2$ and $K_V1.5$ mRNA appeared to start at 24 h (the shortest time tested) and continued to 60 h (the longest time tested) of hypoxia (Fig. 2, *A* and *B*, *right*).

*Effects of hypoxia on mRNA levels of $K_V$ channel β subunits.* In contrast to the inhibitory effect on $K_V1.2$ and $K_V1.5$, hypoxia affected negligibly the mRNA levels of $K_V\beta1$, $K_V\beta2$, and $K_V\beta1$ (Fig. 3). Actually, the mRNA level of $K_V\beta1$ was increased slightly during hypoxia (Fig. 3 *A*), although no significance was observed. These results suggest that the effect of hypoxia on $K_V$ α subunits ($K_V1.2$ and $K_V1.5$) differs from the effect on β subunits in rat PASMC. Hypoxia inhibits gene transcription of $K_V$ α subunits ($K_V1.2$ and $K_V1.5$), but had no effect on gene transcription of $K_V$ β subunits ($K_V\beta1$, $K_V\beta2$, and $K_V\beta3$).

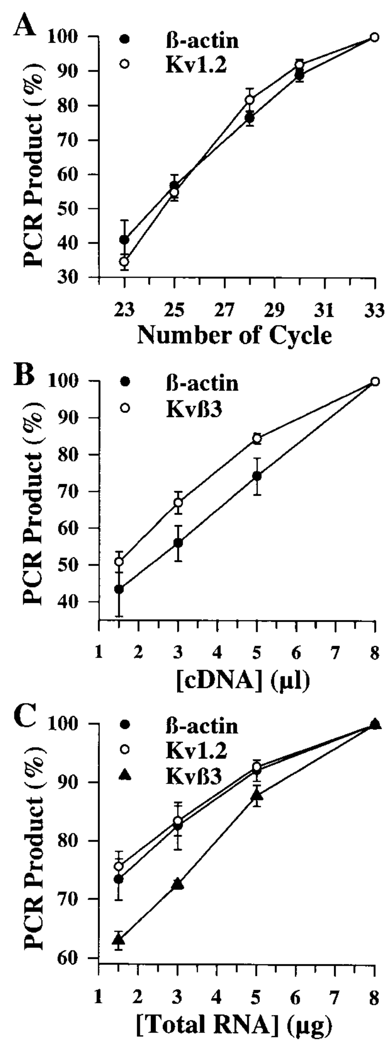


*Figure 1.* Linear relationships between changes in amounts of PCR products and numbers of cycle (*A*), concentrations of cDNA (*B*), or amounts of total RNA (*C*). The data were normalized to the maximal values of the PCR products, and are expressed as means±SEM ($n$ = 4 for each of the experiments).

*Effects of hypoxia on protein levels of $K_V$ channel α subunits.* To confirm that hypoxia-induced inhibition of $K_V$ channel α subunits ($K_V1.2$ and $K_V1.5$) transcription leads to decreased production of the channel proteins, immunoblotting was used to compare protein levels of the channels in PASMC incubated under normoxia and hypoxia. Consistent with the inhibitory effects on transcription of $K_V1.2$ and $K_V1.5$, hypoxia (48–72 h) reduced significantly the amounts of $K_V1.2$ and $K_V1.5$ channel proteins, while the protein level of α-actin was not changed significantly (Fig. 4).

## Discussion

*Molecular characteristics of $K_V$ channel α and β subunits.* The $K_V$ channel is composed of four membrane-bound, pore-forming α subunits and four auxiliary, hydrophilic β subunits (24, 26, 28, 29). There are at least six subfamilies of $K_V$ channel α subunit genes that encode 18 $K_V$ channels: $K_V1.1$–1.7 (*Shaker*), $K_V2.1$–2.2 (*Shab*), $K_V3.1$–3.4 (*Shaw*), $K_V4.1$–4.3 (*Shal*), $K_V5.1$, and $K_V6.1$ (24). $K_V$ channel β subunits were cloned recently from brain ($K_V\beta1.1$–1.3, $K_V\beta2$, and $K_V\beta3$) and heart ($K_V\beta1.1$)

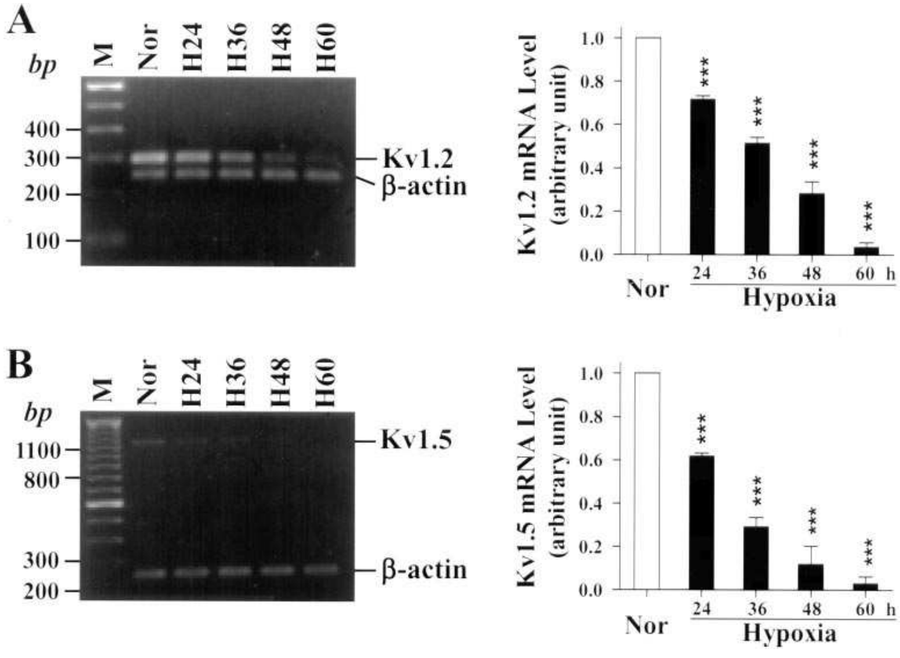


*Figure 2.* Effect of hypoxia on mRNA levels of $K_V$ channel α subunits ($K_V1.2$ and $K_V1.5$) in PASMC. PCR-amplified products are displayed in agarose gels for $K_V1.2$ (295 bp, *A*), $K_V1.5$ (1,111 bp, *B*), and β-actin (244 bp, *A* and *B*), when the first-strand cDNAs, synthesized from total RNA extracted from PASMC incubated in normoxia (*Nor*) and hypoxia for 24 (*H24*), 36 (*H36*), 48 (*H48*), and 60 (*H60*) h, were amplified using the specific sense and antisense primers for β-actin and $K_V$ channel α subunits ($K_V1.2$ and $K_V1.5$; see Table I). *M*, Marker. *Right panels*, Data that were normalized to the amount of β-actin are expressed as means±SEM (experiments were repeated three to four times independently). $^{***}P < 0.001$ vs. normoxic controls (*open bars*).

(25, 28, 29, 33). Association of $K_V$ channel β with α subunits confers the fast A-type inactivation on the slowly or non-inactivating delayed rectifier $K_V$ channels (e.g., $K_V1.2$ and $K_V1.5$) (25, 28). Association of $K_V\beta1$ and $K_V\beta2$ in brain $K^+$ channels is restrictive to $K_V1$ family members; for example, $K_V\beta1$ and $K_V\beta2$ can be associated with $K_V1.1$, $K_V1.2$, $K_V1.4$, and $K_V1.5$, but not with $K_V2.1$ and $K_V4.1$ (25, 26). In addition to changing kinetic properties of $K_V$ α subunits, $K_V$ β subunits can also

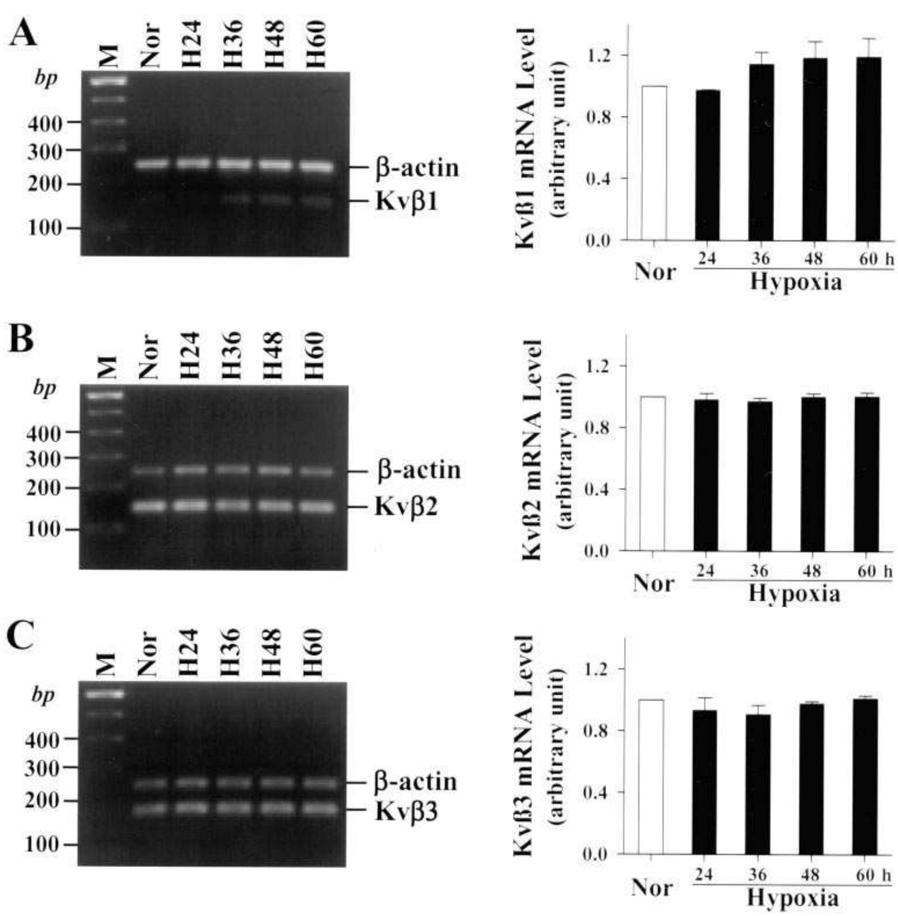


*Figure 3.* Effect of hypoxia on mRNA levels of $K_V$ channel β subunits ($K_V\beta1$, $K_V\beta2$, and $K_V\beta3$) in PASMC. PCR-amplified products are displayed in agarose gels for $K_V\beta1$ (150 bp, *A*), $K_V\beta2$ (141 bp, *B*), $K_V\beta3$ (178 bp, *C*), and β-actin (244 bp, *A*, *B*, and *C*), when the first-strand cDNAs, synthesized from total RNA extracted from PASMC incubated in normoxia (*Nor*) and hypoxia for 24 (*H24*), 36 (*H36*), 48 (*H48*), and 60 (*H60*) h, were amplified using the specific sense and antisense primers for β-actin and $K_V$ channel β subunits ($K_V\beta1$, $K_V\beta2$, and $K_V\beta3$; see Table I). *M*, Marker. *Right panels*, Data that were normalized to the amount of β-actin are expressed as means±SEM (experiments were repeated three to four times independently).

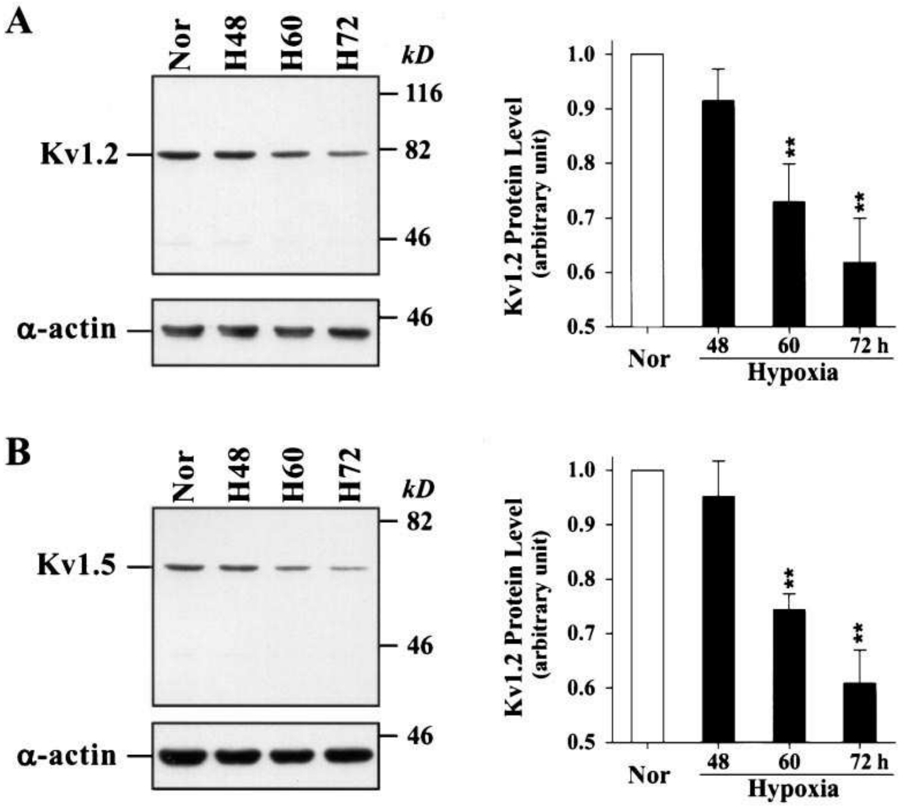


*Figure 4.* Effect of hypoxia on protein levels of $K_V$ channel α subunits ($K_V1.2$, *A*, and $K_V1.5$, *B*) in PASMC. Western blotting analysis of $K_V1.2$ (*A*) and $K_V1.5$ (*B*) channel proteins. Immunoblots of rat PASMC proteins (5 μg/lane) were incubated with affinity-purified anti-$K_V1.2$ (*A*), anti-$K_V1.5$ (*B*), and anti–α-actin (*A* and *B*) antibodies. Molecular mass markers are indicated on right in kilodaltons (kD). The molecular mass of α-actin is ~ 42 kD. Control blot, incubated with rabbit normal serum, was blank and is not shown. *Nor*, Normoxia. *H48*, *H60*, and *H72*, 48, 60, and 72 h of hypoxia, respectively. *Right panels*, Data that were normalized to the amount of α-actin are expressed as means±SEM (experiments were repeated six times independently) during normoxia (*Nor*) and hypoxia (for 48, 60, and 72 h).

block $K_V$ channels and reduce $K_V$ currents as an open-channel blocker (30). The regulatory interaction between α and β subunits and formation of heteromultimeric channels by different membrane-bound α and hydrophilic β subunits contribute significantly to the diversity of native $K_V$ channels and their physiological properties (28, 29).

The time required for reaching a new steady state level of channel activity is dependent on the half-life ($t_{1/2}$) of the channel protein and mRNA. $Na^+$ and $Ca^{2+}$ channels are relatively very stable ($t_{1/2}$ = 1–2 d). The endogenous $K_V$ channels, however, turn over very rapidly, e.g., $t_{1/2}$ for $K_V1.5$ channel protein and mRNA is 4 and 0.5 h, respectively (34). The very short half-life of $K_V$ channels also suggests that the cells undergo rapid exchange of both channel proteins and mRNA under physiological conditions.

*Inhibitory effect of hypoxia on gene transcription of $K_V$ channel α subunits.* Chronic hypoxia increases significantly pulmonary arterial pressure by eliciting pulmonary vasoconstriction and vasoconstruction (15). Pulmonary arterial pressure in animals placed in a hypobaric hypoxic chamber starts to rise at 24 h and is elevated significantly by 48 h of hypoxia. The pressor response is maximized at day 20 of hypoxia, whereas right ventricular hypertrophy occurs after 5 d of hypoxia (15). Thus, in this study, we examined the effect of 24–72 h of hypoxia on gene transcription and expression of $K_V$ channels in PASMC. The data obtained from this study show that: (*a*) hypoxia (24–60 h) inhibited gene transcription of PASMC $K_V$ α subunits ($K_V1.2$ and $K_V1.5$), (*b*) hypoxia (24–60 h) affected negligibly gene transcription of $K_V$ β subunits ($K_V\beta1$, $K_V\beta2$, and $K_V\beta3$) (the $K_V\beta1$ mRNA level was increased slightly), and (*c*) hypoxia (48–72 h) reduced significantly expression of $K_V1.2$ and $K_V1.5$ channel proteins. The results suggest that prolonged hypoxia (~ 72 h) can alter activity of $K_V$ channels by selectively inhibiting gene transcription of $K_V$ channel α subunits.

Amplitude of single-channel $K^+$ current is positively proportional to the channel conductance and the electrochemical driving force. Whole-cell $K^+$ currents ($I_K$) are determined by the following equation:

$$I_K = g_K \times N \times P_{open} \times (E_m - E_K),$$

where $g_K$ is the single-channel conductance, $N$ is the total number of $K^+$ channels, $P_{open}$ is the steady state open probability of $K^+$ channels, $E_m$ is the membrane potential (−40 to −55 mV in PASMC), and $E_K$ is the $K^+$ equilibrium potential (about −85 mV). Transcriptional inhibition of $K_V$ α subunits ($K_V1.2$ and $K_V1.5$) during hypoxia would lead to a decrease in the $K_V$ channel gene products, thereby reducing the number of $K_V$ channels. Unchanged transcription of $K_V$ β subunits during hypoxia may increase the fraction of the $K_V$ channel α subunits that are associated with β subunits (e.g., $K_V1.5$–$K_V\beta1$). Decreased number of $K_V$ channels, along with the blockade effect of $K_V$ β subunits on $K_V$ channel activity (30), would lead to reduction of $I_K$, which has been described in PASMC isolated from chronically hypoxic rats (17, 18). The consequent increase in $[Ca^{2+}]_{cyt}$ (due to $Ca^{2+}$ influx through voltage-gated $Ca^{2+}$ channels and $Ca^{2+}$-induced $Ca^{2+}$ release from intracellular stores) may play an important role in the development of pulmonary vasoconstriction and vascular remodeling (5–7).

*Possible mechanisms involved in hypoxia-induced inhibition of $K_V$ channel transcription.* Reduced $O_2$ tension has a significant influence on gene regulation in a variety of tissues and cells. The cell signaling pathways by which hypoxia regulates gene transcription and translation appear to be very complex (35, 36). In pulmonary vascular endothelial cells, hypoxia inhibits expression of nitric oxide synthase (eNOS) by suppressing the transcriptional rate of the eNOS gene and decreasing the half-life ($t_{1/2}$) of the eNOS mRNA (37). In PASMC, hypoxia also decreases mRNA transcripts of ornithine decarboxylase and *S*-adenosylmethionine decarboxylase (38). Distinct action of various oxygen radicals generated by hemoproteins (e.g., cytochrome, or NADPH oxidoreductase) as a function of changes in $O_2$ tension and cellular redox state may be involved in the down- or upregulation of gene transcription (35, 36, 39–41). The main molecular pathways appear to be the modification of $O_2$-regulated transcriptional factors that subsequently turn on or off the target genes (36). It has been demonstrated recently that the hypoxia-inducible factor 1 (HIF-1) plays an important role in inducing gene transcription of $O_2$-dependent proteins, such as erythropoietin (41, 42). Chronic exposure to hypoxia (~ 3 d) increases HIF-1 DNA–binding activity in isolated rat lungs (43) and in cultured bovine PASMC (44). Whether HIF-1 is related to induction of an intermediate mediator that downregulates gene transcription of $K_V$ channels during hypoxia has not yet been elucidated.

The precise cellular and molecular mechanisms through which hypoxia inhibits mRNA levels of $K_V1.2$ and $K_V1.5$ (e.g., whether it is due to decreased transcriptional rate or changes in mRNA stability) are unknown. It may be related to the cellular redox state, hemoprotein (e.g., cytochrome, or NADPH oxidoreductase) activity, and reactive oxygen intermediates (35–37, 39–42, 45). Hypoxia alters cellular redox status and inhibits the heme-containing proteins (1, 10, 39–42). Thus, it is reasonable to speculate that the hypoxia-induced inhibition of heme- and/or metal-containing enzymes may serve as an intermediate to downregulate gene transcription of $K_V$ channels (39–42).

*Summary and conclusions.* The results from this study demonstrate that prolonged hypoxia downregulates gene transcription and expression of $K_V$ channel $\alpha$ subunits ($K_V1.2$ and $K_V1.5$), but affects negligibly transcription of $K_V$ channel $\beta$ subunits ($K_V\beta1$, $K_V\beta2$, and $K_V\beta3$) in rat PASMC. The consequent decrease in the number of $K_V$ channels would lead to decreased $K_V$ currents, due to a reduction in current availability (17), and depolarized membrane potential, which have been observed in PASMC isolated from chronically hypoxic animals (17, 18). The hypoxia-mediated transcriptional regulation of $K_V$ channel genes in PASMC may play a causal role in the development of HPV and pulmonary hypertension during chronic hypoxia.

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