Inhibition of K⁺ Channel Activity in Human Pulmonary Artery Smooth Muscle Cells by Serum from Patients with Pulmonary Hypertension Secondary to Congenital Heart Disease

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ABSTRACT

Activity of K⁺ channels regulates cytosolic free Ca²⁺ concentration by controlling membrane potential. A rise in cytosolic free Ca²⁺ concentration in pulmonary artery smooth muscle cells (PASMC) triggers pulmonary vasoconstriction and stimulates PASMC proliferation. Whether serum from children with pulmonary hypertension (PH) secondary to congenital cardiopulmonary diseases contains a factor(s) that inhibits K⁺ channel function in PASMC was investigated using patch clamp techniques. PASMC isolated from normal subjects were cultured in media containing 5% serum from normotensive (NPH) or PH patients. Cell growth rate and the currents through voltage-gated K⁺ channels were determined and compared between the cells treated with serum from NPH and PH patients. In the absence of growth factors, incubation of PASMC in media containing NPH serum for 48 h increased cell numbers by 2.5-fold, whereas incubation of the cells in media containing PH serum increased cell numbers by 4.5-fold ($p < 0.001$). Amplitude of whole-cell voltage-gated K⁺ currents in NPH serum-treated cells (1119 ± 222 pA at +80 mV, $n = 43$) was 3.5-fold greater than in PH serum-treated cells (323 ± 34 pA, $n = 43$, $p < 0.001$). Consistently, membrane potential was much more depolarized in PASMC treated with PH serum (-28 ± 2 mV, $n = 29$) than cells treated with NPH-serum (-47 ± 2 mV, $n = 28$; $p < 0.001$).

These results suggest that a circulating mitogenic agonist, which induces membrane depolarization by inhibiting voltage-gated K⁺ channel activity in PASMC, may be produced or up-regulated in pediatric patients with secondary PH. (*Pediatr Res* 50: 23–28, 2001)

Abbreviations

[Ca²⁺]_{cyt}, cytosolic free Ca²⁺ concentration
 E_m , membrane potential
FBS, fetal bovine serum
hEGF, human epidermal growth factor
hFGF, human fibroblast growth factor
 $I_{K(V)}$, whole-cell voltage-gated K⁺ currents
Kv, voltage-gated K⁺ channels
NPH, non-pulmonary hypertension cardiopulmonary disease
PAP, pulmonary arterial pressure
PASMC, pulmonary artery smooth muscle cells
PH, pulmonary hypertension
PPH, primary pulmonary hypertension
PSS, physiologic salt solution
SMBM, smooth muscle basal medium
SMGM, smooth muscle growth medium
VEGF, vascular endothelium growth factor

PH contributes significantly to the morbidity and mortality of children with congenital heart defects (1). PH in congenital heart disease may be caused by increased pulmonary blood flow, left-side obstructive lesions, pulmonary vasoconstriction, vascular remodeling, and thrombosis *in situ*. These causes can

be present in isolation or in combination (2). The most common cardiac defect associated with PH is a ventricular septal defect. If the defect is left open or remains large, many patients develop pulmonary vascular obstructive disease and irreversible PH (3); this phenomenon is called Eisenmenger syndrome. Patients with Eisenmenger syndrome have elevated pulmonary vascular resistance and systemic or suprasystemic PAP (which causes a right-to-left shunt).

The pathophysiology of PH secondary to congenital heart lesions shares several characteristics with PPH; including persistent pulmonary vasoconstriction and significant vascular wall remodeling (mainly because of smooth muscle hypertro-

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phy and proliferation) (1). However, the precise cellular mechanisms leading to PH in children with congenital heart defects remain incompletely understood. Recently, we observed that expression and function of Kv channels in PASMC are both attenuated in patients with PPH, suggesting that dysfunction of Kv channels may play an important role in the development of PH in patients with PPH (4, 5).

Activity of Kv channels regulates resting E_m in PASMC (6–8). E_m , by controlling the function of sarcolemmal voltage-gated Ca^{2+} channels, is a key determinant of $[Ca^{2+}]_{cyt}$ and thus pulmonary vascular tone (7, 9, 10). Inhibition of Kv channels results in membrane depolarization, which opens voltage-dependent Ca^{2+} channels, promotes Ca^{2+} influx, and increases $[Ca^{2+}]_{cyt}$ in PASMC (6–12). A rise in $[Ca^{2+}]_{cyt}$ has been demonstrated to be a major trigger for vasoconstriction (10) and an important stimulus for cell proliferation (13, 14). Accordingly, this study was designed to test the hypothesis that the serum from children with PH secondary to congenital heart defects may possess a factor(s) that stimulates cell proliferation by inhibiting K^+ channel activity in normal PASMC.

METHODS

Serum preparation. Patients participating in this study were classified into two groups based on hemodynamic data (Table 1): 1) normotensive control group including children with normal PAP; and 2) PH group including children with a mean PAP $\geq 40\%$ of mean systemic arterial pressure. Blood (3–5 mL) was obtained from each patient at the time of cardiac catheterization. The serum was isolated from whole blood and used to perform the proposed experiments. The study was approved by the Human Research Committee of the University of California, San Diego. Informed consent was obtained from all participant patients before blood was drawn.

Cell culture. Human PASMC from normal subjects were purchased from Clonetics (BioWhittaker Inc., Walkersville, MD, U.S.A.) and used at the fourth to sixth passages. The cells, plated onto coverslips (for electrophysiologic experiments) or Petri dishes (for cell counting), were first cultured in SMGM

(Clonetics) in a 37°C, 5% CO_2 , humidified incubator for 48–60 h. The SMGM consisted of SMBM, 5% FBS, 5 μ g/mL insulin, 2 ng/mL hFGF, and 0.5 ng/mL hEGF (Clonetics). The proliferating PASMC cultured in SMGM were then growth-arrested by culturing them in SMBM for 24–48 h before each experiment.

The purity of PASMC in cultures was confirmed by the specific MAb raised against smooth muscle α -actin (Boehringer-Mannheim Biochemicals, Indianapolis, IN, U.S.A.). The cells were stained with the membrane-permeable nucleic acid stain, 4',6'-diamidino-2-phenylindole (DAPI, 5 μ M; Molecular Probes, Eugene, OR, U.S.A.). All the DAPI-stained cells in the cultures cross-reacted with the smooth muscle cell α -actin antibody, indicating that the cultures consisted only of smooth muscle cells. There were no significant morphologic differences in the cells cultured in media with and without serum or growth factors.

Treatment of cells with serum from NPH or PH patients. Quiescent (growth-arrested) PASMC cultured in SMBM were divided into two groups. One group of cells was cultured in SMBM containing 5% serum from NPH patients (with or without the growth factors). The other group was cultured in SMBM containing 5% serum from PH patients (with or without the growth factors). Both groups of cells were incubated in an incubator equilibrated with 5% CO_2 in air. After 48 h, the cell growth rate was determined using a hemocytometer, and whole-cell I_K were measured by patch clamp techniques.

Determination of growth rate. Cells attached to Petri dishes were trypsinized and harvested into 10-mL tubes. After 5 min of centrifugation at 15,000 rpm, the cell pellet was resuspended into Hanks' balanced salt solution (Clonetics). Nine microliters of cell suspension was pipetted into one of the counting chambers in a hemocytometer. Viable cell count was determined in each of the four 1-mm³ corner squares and averaged to calculate total cell number (cells/milliliter) in cell suspension. The normalized cell number by size of the Petri dishes (cells/square centimeter) was used to compare cell growth rate.

Table 1. Demographic, clinical, and hemodynamic characteristics of study sample

Patient No.	Sex	Age	Diagnosis	mPAP (mm Hg)	mSBP (mm Hg)	$\dot{Q}_p/\dot{Q}_s$	[Hb] (mg/dL)	$S\bar{v}O_2$ (mm Hg)
NPH patients								
1	M	10 y	D-TGA/VSD and straddling MV S/P Fontan	11	104	1	12.0	66
2	M	7 y	ASD	14	81	1.3	11.8	78
3	M	9 y	DORV and hypoplastic LV S/P Fontan	13	59	0.7	14.4	69
4	M	2 mo	TOF and PDA	NA*	40	~1.5	11.0	61
5	F	1 y	TOF/PA S/P bilateral unifocalization and central shunt	13	48	1.7	14.8	61
6	F	1 y	PDA	11	53	~1.6	—	52
7	F	9 y	TAPVR/ASD and valvar PS	8	69	>3.0	14.6	66
PH patients								
8	M	3.5 y	VSD/CoA/subAS/PDA	58	58	2.6	11.2	76
9	M	2 mo	VSD	32	45	>3.0	10.0	56
10	M	6 y	Diaphragmatic hernia/ASD	94	72	0.7	14.0	79

mPAP, mean pulmonary artery pressure; mSBP, mean systemic blood pressure; $\dot{Q}_p/\dot{Q}_s$, the ratio of pulmonary blood flow to systemic blood flow; $S\bar{v}O_2$, mixed venous oxygen saturation; TGA, transposition of the great arteries; VSD, ventricular septal defect; MV, mitral valve; S/P, status post; PA, pulmonary atresia; LV, left ventricle; ASD, atrial septal defect; DORV, double-outlet right ventricle; TOF, tetralogy of Fallot; PDA, patent ductus arteriosus; CoA, coarctation of the aorta; AS, aortic stenosis; TAPVR, total anomalous pulmonary venous return; PS, pulmonary stenosis.

* Hemodynamic measurements not available.

Cell viability was determined using 0.45% trypan blue (Sigma Chemical Co., St. Louis, MO, U.S.A.).

Electrophysiologic measurements. Whole-cell I_K were recorded with an Axopatch-1D amplifier and a DigiData 1200 interface (Axon Instruments, Burlingame, CA, U.S.A.) using patch clamp techniques (7, 15). Patch pipettes (2–4 M Ω) were made on a Sutter electrode puller (Sutler Instrument Co., Novato, CA, U.S.A.) using borosilicate glass tubes and fire polished on a Narishige microforge (Narishige Scientific Instrument Lab, Tokyo, Japan). Step-pulse protocols and data acquisition were performed using pCLAMP software. Currents were filtered at 1–2 kHz (–3 dB) and digitized at 2–4 kHz using an Axopatch-1D amplifier. All experiments were performed at room temperature (22–24°C). E_m in single PASM C was measured in current-clamp mode ($I = 0$) using whole-cell patch clamp techniques.

Reagents and solutions. A coverslip containing the cells was positioned in the recording chamber (approximately 0.75 mL) on a Nikon microscope and superfused (2 mL/min) with the extracellular (bath) PSS for recording I_K . The PSS contained (in mM): 141 NaCl, 4.7 KCl, 1.8 CaCl₂, 1.2 MgCl₂, 10 HEPES, and 10 glucose (pH 7.4). In Ca²⁺-free PSS, CaCl₂ was replaced by equimolar MgCl₂ and 1 mM EGTA was added to chelate residual Ca²⁺. The internal (pipette) solution for recording $I_{K(V)}$ contained (mM): 125 KCl, 4 MgCl₂, 10 HEPES, 10 EGTA, 5 Na₂ATP (pH 7.2).

Statistical analysis. The composite data are expressed as mean $\pm$ SEM. Statistical analyses were performed using unpaired t test or ANOVA and *post hoc* tests (Student-Newman-Keuls) as appropriate. Differences were considered to be significant when $p < 0.05$.

RESULTS

Demographic and clinical characteristics of patients. The sera used for this study were obtained from five NPH patients and three PH patients. The clinical, demographic, and hemodynamic characteristics of the eight subjects are shown in Table 1. The average mean PAP in PH patients was significantly higher than in NPH patients (50.7 ± 6.4 versus 11.7 ± 2.2 mm Hg, $p < 0.01$), whereas the mean systemic arterial pressure was similar (60.3 ± 7.7 versus 64.8 ± 21.9 mm Hg, $p = 0.5$). Furthermore, the ratio of pulmonary blood flow to systemic blood flow in NPH patients was 1.5 ± 0.7 and in PH patients was 2.8 ± 0.2 ($p < 0.01$). These results indicate that the PH patients have high PAP and high pulmonary blood flow.

Enhanced cell proliferation in PASM C treated with serum from PH patients. PASM C were first growth-arrested in media without serum or growth factors. After 24 h, the quiescent PASM C were then cultured in media containing 5% serum from NPH patients or PH patients in the absence or presence of growth factors (*i.e.* insulin, hFGF, and hEGF) for 48 h. As shown in Figure 1, increases in cell numbers after 48 h were significantly greater in PASM C cultured in media containing serum from PH patients (from 1820 ± 244 to 9879 ± 1012 cells/cm²) than in cells cultured in media containing serum from NPH patients (from 1820 ± 244 to 6462 ± 494 cells/

cm²). These results indicate that the growth rate in cells treated with PH serum was 1.75-fold greater than in cells treated with NPH serum. Addition of growth factors (*e.g.* insulin, hFGF, and hEGF) appeared to have little effect on cell growth in PASM C cultured in media containing 5% serum from NPH or PH patients (Fig. 1). These observations suggest that the serum from PH patients may include a mitogenic factor that stimulates PASM C growth in the presence or absence of insulin, hFGF, and hEGF.

Reduced whole-cell $I_{K(V)}$ in PASM C treated with serum from PH patients. Whole-cell $I_{K(V)}$ were isolated in PASM C superfused with Ca²⁺-free (plus 1 mM EGTA) bath solution and dialyzed with Ca²⁺-free (plus 10 mM EGTA) and ATP-containing pipette solutions. The removal of extracellular and intracellular Ca²⁺ and inclusion of 5 mM ATP in the pipette solution minimized the contribution of Ca²⁺-activated and ATP-sensitive K^+ currents to the measured currents.

Whole-cell $I_{K(V)}$ were elicited by depolarizing the cells from a holding potential of –70 mV to a series of test potentials ranging from –80 to +80 mV in increments of 20 mV (Fig. 2A). The amplitude of the steady-state $I_{K(V)}$ in PASM C was significantly attenuated by incubating the cells in media containing serum from PH patients, in comparison with the cells cultured in media containing serum from NPH patients (Fig. 2, A and B). The composite current-voltage relationships (I - V curves) demonstrate that PH serum caused an approximately 71% reduction of whole-cell $I_{K(V)}$ amplitude at +80 mV (from 1119 ± 222 to 323 ± 34 pA, $p < 0.001$) and an approximately 81% decrease at –20 mV (from 40 ± 8 to 8 ± 2 pA, $n = 43$, $p < 0.001$; Fig. 2B). The averaged currents, elicited by a +80-mV test pulse, show that the activation and inactivation kinetics of the currents were unaltered in PASM C treated with PH serum, in comparison with the cells cultured in media containing NPH serum (Fig. 2C). The time constants for current activation were 3.4 ± 0.5 and 3.6 ± 0.5 ms ($p = 0.73$)

Figure 1. Enhancement of PASM C growth by serum from PH patients. Cell numbers were determined using a hemacytometer before (basal) and after treatment of the cells with serum from NPH or PH patients in the absence (–GF) or presence (+GF) of growth factors (GF). Data are mean $\pm$ SEM with numbers of independent experiments in parentheses. ** $p < 0.01$, *** $p < 0.001$ vs NPH.

Figure 2. Inhibitory effect of serum from PH patients on amplitudes of whole-cell $I_{K(V)}$ in PASC. *A*, representative families of currents, elicited by depolarizing the cells to a series of test potentials ranging from -60 to $+80$ mV in 20 -mV increments (holding potential, -70 mV), in PASC cultured in serum from NPH or PH patients. *B*, current-voltage relationships ($I-V$ curves) of the steady-state $I_{K(V)}$ in PASC treated with NPH serum ($\circ$, $n = 43$) and PH-serum ($\bullet$, $n = 43$). Data are mean $\pm$ SEM. The $I-V$ curve of NPH is significantly different from the $I-V$ curve of PH ($p < 0.001$, Student-Newman-Keuls). *C*, averaged currents at $+80$ mV from PASC treated with NPH or PH serum. *D*, kinetics of the current activation (*a*) and inactivation (*b*) in PASC treated with serum from NPH ($\circ$) or PH (gray squares) patients. The currents, elicited by $+80$ mV (holding potential, -70 mV), were averaged from all of the cells tested and normalized to the maximal amplitude of each of the current records. *E*, summarized data (mean $\pm$ SEM) showing the time-constants (τ) for current activation (*a*) and inactivation (*b*) in PASC treated with NPH or PH serum.

and for current inactivation, 125.1 ± 37.3 and 160.7 ± 35.8 ms ($p = 0.53$) in PASC treated with NPH or PH serum, respectively (Fig. 2, *D* and *E*).

Consistent with the inhibitory effect on whole-cell $I_{K(V)}$, the serum from PH patients also caused a significant membrane depolarization in PASC (Fig. 3). The average resting E_m in PASC cultured in media containing NPH serum was -47 ± 2 mV ($n = 28$), whereas in cells cultured in media containing PH serum it was -28 ± 2 mV ($n = 29$, $p < 0.001$). These observations indicate that serum from PH patients may contain a K^+ channel antagonist that blocks K_v channels. The resultant decrease in the amplitude of $I_{K(V)}$ induces membrane depolarization and may thus increase $[Ca^{2+}]_{cyt}$ by activating voltage-gated Ca^{2+} channels in PASC (7, 9).

These observations demonstrate that, in PASC treated with serum from PH patients, 1) K_v channel activity was diminished, 2) E_m was depolarized, and 3) cell proliferation rate was enhanced, in comparison with cells treated with serum from NPH patients. The next set of experiments was designed to examine whether decreased K_v channel activity and membrane depolarization were involved in human PASC proliferation by comparing $I_{K(V)}$ and E_m in growth-arrested and proliferating PASC.

Comparison of whole-cell $I_{K(V)}$ and resting E_m in growth-arrested and proliferating PASC. Human PASC were first plated in Petri dishes and cultured in the growth medium, SMGM (containing 5% FBS and growth factors). After 24 h, the cells were divided into two groups and cultured in SMGM (growth medium) and SMBM (basal medium without serum and growth factors) for 48–60 h. Removal of serum and growth factors from culture medium significantly inhibited human PASC growth (Fig. 4*A*), suggesting that cells cultured in serum-free medium (SMBM) are growth-arrested cells, and cells cultured in serum- and growth factor-containing medium (SMGM) are proliferating cells.

In proliferating PASC, amplitude of whole-cell $I_{K(V)}$ was significantly reduced, in comparison with growth-arrested cells (Fig. 4, *B* and *C*). Accordingly, resting E_m in proliferating PASC was much more depolarized than in growth-arrested PASC (Fig. 4*D*). In smooth muscle cells, the voltage window of sarcolemmal L-type voltage-gated Ca^{2+} channels for sus-

Figure 3. Effect of serum from PH patients on resting E_m in PASC. E_m was measured in cells cultured in media containing 5% serum from NPH or PH patients. The gray and solid circles represent individual E_m values measured from the cells, and the boxes depict the composite values (mean $\pm$ SD) of E_m in PASC treated with serum from NPH or PH patients.

Figure 4. Comparison of cell growth rate, whole-cell $I_{K(V)}$, and resting E_m between growth-arrested and proliferating PASC. *A*, cell numbers (mean $\pm$ SEM) were determined using a hemacytometer before and after 2-d incubation in SMBM or SMGM. $***p < 0.01$ (Student-Newman-Keuls) vs SMBM. *B*, representative families of currents, elicited by depolarizing the cells from a holding potential of -70 mV to a series of test potentials ranging from -40 mV to $+80$ mV in 20 -mV increments, in growth-arrested (cultured in SMBM) and proliferating (cultured in SMGM for 2–3 d) PASC. *C*, current-voltage relationships (I - V curves) of steady-state $I_{K(V)}$ in PASC cultured in SMBM (growth-arrested cells) and SMGM (proliferating cells). Data are mean $\pm$ SEM. The I - V curve of growth-arrested cells is significantly different from the I - V curve of proliferating cells ($p < 0.001$, Student-Newman-Keuls). *D*, resting E_m measured in current-clamp mode ($I = 0$) in growth-arrested (SMBM) and proliferating (SMGM) PASC. Data are mean $\pm$ SEM. $***p < 0.001$ vs SMBM.

tained elevation of $[Ca^{2+}]_{cyt}$ ranges from -40 to -20 mV and peaks at -30 mV (11). Thus, the sustained membrane depolarization in proliferating PASC may produce a constant Ca^{2+} influx through L-type voltage-gated Ca^{2+} channels, and contribute to initiating and maintaining the elevated $[Ca^{2+}]_{cyt}$ crucial for cell proliferation (7, 9, 11, 14).

DISCUSSION

The precise cellular mechanisms responsible for the elevated pulmonary vascular resistance and arterial pressure in patients

with congenital cardiopulmonary diseases remain incompletely understood. An increased circulating factor that induces pulmonary vasoconstriction and stimulates PASC and endothelial cell growth has been implicated in patients with PH (16–20). The observations from this study demonstrated that the serum from PH patients 1) significantly enhanced normal PASC proliferation in the absence or presence of growth factors, 2) markedly decreased the amplitude of whole-cell $I_{K(V)}$, and 3) significantly depolarized E_m in PASC. Furthermore, $I_{K(V)}$ was greatly decreased and E_m was significantly depolarized in proliferating cells compared with growth-arrested cells, indicating a close linkage between membrane depolarization (owing to decreased K^+ channel activity) and cell proliferation in human PASC (14).

Serum from patients with PH secondary to congenital heart defects may contain 1) a vasoactive agonist that inhibits Kv channels, induces E_m depolarization, opens voltage-dependent Ca^{2+} channels, increases $[Ca^{2+}]_{cyt}$, and eventually stimulates cell growth; 2) a mitogenic agonist that stimulates cell growth by activating a Kv channel-independent pathway; and 3) both vasoactive and mitogenic agonists. The mitogenic agonist(s) or Kv channel blocker(s) may be produced sporadically as a result of high blood flow and arterial pressure (e.g. shear stress-mediated endothelium injury) (21–23). Another possibility is that the existing mitogenic agonists in serum are up-regulated in patients with PH secondary to congenital cardiopulmonary diseases.

Shear stress, caused by hyperkinetic pulmonary circulation in patients with PH secondary to congenital heart defects, may cause endothelial injury and lead to release of a variety of vasoconstrictor agonists, e.g. endothelium-derived constricting factors (23), cytokines (e.g. interleukins, leukotrienes) (16), and serotonin (17). Shear stress-mediated gene expression of growth factors (e.g. VEGF) in endothelial cells and their release to the pulmonary circulation (24) may also contribute to mediating pulmonary vasoconstriction and vascular remodeling in patients with PH. Stretch of the pulmonary vessels, owing to high blood flow and arterial pressure, increases activity of stretch-activated cation channels and facilitates synthesis and release of vasoactive substances and mitogenic agonists by raising $[Ca^{2+}]_{cyt}$ in vascular smooth muscle and endothelial cells. Furthermore, the hypoxemia and ischemia caused by congenital heart defects, e.g. Eisenmenger syndrome, may also facilitate release and production of vasoactive and mitogenic agonists (e.g. norepinephrine, epinephrine, serotonin, VEGF).

A variety of vasoactive and mitogenic substances are present in the serum of children with PH secondary to congenital heart disease (1, 3, 20, 24). Increased blood levels of endothelin-1 (an endothelium-derived constricting factor) (18–22), VEGF (24), serotonin (17), and angiotensin (25) have been implicated in the development of PH. Up-regulated gene expression of angiotensin and its release to the blood from cardiac myocytes may also contribute to the pulmonary vascular remodeling in patients with PH secondary to congenital heart defects [(25); also Dr. P.A. Thistlethwaite, personal communication]. The results from the current study imply that the up-regulated mitogenic substances (e.g. endothelin-1) in pa-

tients with congenital heart diseases may stimulate PASM C proliferation by, at least partially, attenuating Kv channel activity in PASM C.

The observations shown in Figure 2 indicate that serum from PH patients only decreased the amplitude of $I_{K(V)}$ with no effect on the kinetics of channel activation and inactivation. The amplitude of whole-cell $I_{K(V)}$ is proportional to the number of functional Kv channels expressed in the plasma membrane and the amplitude of the unitary current (which is determined by the single-channel conductance and the steady-state open probability of the Kv channel). Therefore, the cellular mechanisms responsible for the inhibitory effect of PH serum on whole-cell $I_{K(V)}$ may likely result from down-regulation of gene expression of Kv channel α subunits, the pore-forming subunits, and decrease in $I_{K(V)}$ availability. The resultant membrane depolarization promotes Ca^{2+} influx by opening voltage-dependent Ca^{2+} channels, a major pathway for Ca^{2+} influx in vascular smooth muscle cells (9, 11). A rise in $[Ca^{2+}]_{cyt}$, which rapidly increases nuclear $[Ca^{2+}]$ (within 200 ms) (26) and gradually increases the intracellularly stored $[Ca^{2+}]$ in the sarcoplasmic reticulum (27), can 1) activate mitogen-activated protein (MAP) kinase cascade that stimulates synthesis of transcription factors required for cell proliferation, and 2) promote cell proliferation by moving quiescent cells into the cell cycle and by propelling the proliferating cells through mitosis (28–31).

In summary, the results from this study suggest that a circulating Kv channel blocker may be produced or a mitogenic agonist existing in serum may be up-regulated in patients with PH secondary to congenital cardiopulmonary diseases. The agonist-mediated inhibition of Kv channels in PASM C induces membrane depolarization, which may increase $[Ca^{2+}]_{cyt}$ by opening L-type voltage-dependent Ca^{2+} channels. The increase in $[Ca^{2+}]_{cyt}$ in PASM C then triggers cell contraction by activating calmodulin and stimulates cell proliferation by accelerating the progression of the cell cycle. The resultant pulmonary vasoconstriction and vascular medial hypertrophy ultimately contribute to increase pulmonary vascular resistance and arterial pressure.

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