Rat pulmonary arterial smooth muscle myosin light chain kinase and phosphatase activities decrease with age

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Belik, J., Ewa Kerc, and Mary D. Pato. Rat pulmonary arterial smooth muscle myosin light chain kinase and phosphatase activities decrease with age. Am J Physiol Lung Cell Mol Physiol 290: L509–L516, 2006—We and others have shown that the fetal pulmonary arterial smooth muscle potential for contraction and relaxation is significantly reduced compared with the adult. Whether these developmental changes relate to age differences in the expression and/or activity of key enzymes regulating the smooth muscle mechanical properties has not been previously evaluated. Therefore, we studied the catalytic activities and expression of myosin light chain kinase (MLCK) and myosin light chain phosphatase (MLCP) catalytic (PP1c) and regulatory (MYPT) subunits in late fetal, early newborn, and adult rat intrapulmonary arterial tissues. In keeping with the greater force development and relaxation of adult pulmonary artery, Western blot analysis showed that the MLCK, MYPT, and PP1c contents increased significantly with age and were highest in the adult rat. In contrast, their specific activities (activity/enzyme content) were significantly higher in the fetal compared with the adult tissue. The fetal and newborn pulmonary arterial muscle relaxant response to the Rho-kinase inhibitor Y-27632 was greater than the adult tissue. In addition to the 130-kDa isoform of MLCK, we documented the presence of minor higher-molecular-weight embryonic isoforms in the fetus and newborn. During fetal life, the lung pulmonary arterial MLCK- and MLCP-specific activities are highest and appear to be related to Rho-kinase activation during lung morphogenesis.

VASCULAR RESISTANCE IS dynamically controlled by the contraction and relaxation of its smooth muscle layer. Although this process is modulated by a number of humoral and nonhumoral factors, the vessel maximum potential for constriction and dilation is constrained by the vascular smooth muscle mechanical properties. We have previously shown that the pulmonary and systemic vascular smooth muscle of the newborn sheep has a lesser potential for force development, and a longer relaxation half-time compared with the adult (6, 9). Similar age-related systemic vascular smooth muscle differences have been reported in relation to its maximum contraction in the rabbit (27) and rat (22), as well as to the degree of nitric oxide-induced relaxation in the guinea pig (5).

The smooth muscle mechanical properties are primarily dependent on the rates of phosphorylation and dephosphorylation of the 20-kDa light chains of myosin by myosin light chain kinase (MLCK) and myosin light chain phosphatase (MLCP), respectively. These enzymes are key smooth muscle proteins that regulate contraction and relaxation, respectively. We have previously reported that prenatally induced pulmonary hypertension is associated with a significant reduction in pulmonary arterial MLCK and MLCP content (8). These findings correlate with the reduced pulmonary arterial smooth muscle potential for contraction and longer half-time relaxation, suggesting that these alterations contribute to the maintenance of a high pulmonary vascular resistance in pulmonary hypertension.

Limited data are available concerning the MLCP and MLCK pulmonary vascular developmental changes in mammals. There are several reasons to suggest that the activity of these enzymes in the pulmonary arterial tissue is age dependent. The first relates to evidence that distinct MLCK isoforms (208–220 and 130 kDa) have been identified in various tissues, with the higher molecular embryonic forms predominating early in life (10, 16). Secondly, the regulatory subunit of MLCP (MYPT) has been shown to be developmentally regulated in chicken gizzard (12). Thirdly, phosphorylation of MYPT by Rho-kinase has been described (21). Aside from being important for lung morphogenesis during fetal life (24) the Rho-kinase-dependent MLCP inhibition results in Ca2+ sensitivity-dependent increase in smooth muscle contraction, as well as reduced relaxation. Evidence for the developmental regulation of this process is demonstrated in porcine pulmonary arterial tissue where the extent of hypoxia-induced Rho-kinase activation was reported to be age dependent (4).

Therefore we studied the late fetal, early newborn, and adult rat 3rd- to 4th-generation intrapulmonary arterial smooth muscle mechanical properties and MLCK and MLCP protein content and activities. Our results showed age-dependent changes in the pulmonary vascular smooth muscle mechanical properties. These changes were mirrored by a developmental increase in MLCK and MLCP vascular protein content.

METHODOLOGY

Animal preparation. We removed late-gestation fetal (20–21 days, n = 40), newborn (1–3 days, n = 32), and adult (n = 44) Sprague-Dawley rats’ lungs and/or aorta immediately after killing the animals with an overdose of pentobarbital sodium (50 mg/kg). The lungs were dissected to isolate and remove the 3rd- to 4th-generation intrapulmonary arteries. Given their small size, each fetal and newborn pulmonary artery sample used for the biochemical studies comprised pool tissue from three animals, and the sample size (n) reflects the number of tissue pools. The tissue was immediately frozen on liquid nitrogen for the biochemical measurements or freshly mounted on a myograph for mechanical studies.

This protocol was approved by the Hospital for Sick Children Research Institute Committee on Animal Experimentation.

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Preparation of tissue extracts. The frozen tissue was ground and homogenized in the extraction buffer (0.5 mM NaCl, 50 mM Tris-HCl, pH 7.5, 10 mM MgCl₂, 1 mM EDTA, 1 mM EGTA, 5 mM diithiothreitol) in the presence of protease inhibitors (0.1 mM phenylmethylsulfonyl fluoride, 10 mg/ml benzylarginine methyl ester, 10 mg/ml soybean trypsin inhibitor, 1 mg/ml leupeptin, 1 mg/ml pepstatin, and 10 mg/ml 1-1-tosylamido-2-phenylethyl chloromethyl ketone) at 4°C for 1 h. The homogenate was centrifuged in a microfuge at 15,000 rpm for 15 min at 4°C, and the supernatant of the extract was assayed for enzyme activities. The protein content of the extract was determined by the method of Bradford (11).

MLCK activity assay. MLCK activity was determined in a reaction mixture (100 µl) containing 50 mM Tris-HCl (pH 7.5), 3.1 mg/ml 20-kDa turkey gizzard myosin light chains, 0.1 mM [32P]ATP, 0.2 mM MgCl₂, 10 mM MgCl₂, and 0.1 µM calmodulin at 24°C, as previously described (8). The reaction was initiated by the addition of the extract. The time course of the reaction was determined by taking 20-µl aliquots of the reaction mixture at various times and spotted on strips of P81 phosphocellulose paper. The paper strips were washed with 75 mM H₂PO₄, dried, added to 4 ml of scintillation fluid, and counted in a liquid scintillation counter. The MLCK activity (nmol [32P]PO₄ incorporated min⁻¹ mg⁻¹ protein in tissue extract) was calculated from the linear portion of the activity curve with respect to time. No significant Ca²⁺-independent protein kinase activity and Ca²⁺-calmodulin dependent protein kinase II activity were observed as determined by performing the assay in the presence of EGTA, and Ca²⁺, and KN-92, respectively.

MLCP activity assay. MLCP activity was monitored by the release of [32P]PO₄⁻ from the substrate, as previously described (8). The substrate used was [32P]PO₄⁻ labeled myosin heavy meromyosin (HMM) prepared from turkey gizzards and phosphorylated by MLCK. HMM is a chymotryptic fragment of myosin that retains the structure of the head region and its actin-activated MgATPase activity but cannot polymerize due to the loss of a part of its tail region. As a consequence, HMM is soluble at high concentration in low ionic strength solutions. Under these conditions, myosin precipitates out and is not suitable for the assay of MLCP activity. The dephosphorylation reaction was initiated by addition of tissue extract to a reaction mixture (total volume of 200 µl) containing 50 mM Tris-HCl (pH 7.4), 0.5 mM myosin HMM, and 1 mM diithiothreitol at 30°C. We determined the time course of the reaction by taking 40-µl aliquots of the reaction mixture at various times and pipetting them into 100 µl of 17.5% trichloroacetic acid to terminate the reaction. After the addition of 100 µl of 6 mg/ml of bovine serum albumin, the resulting mixture was chilled and centrifuged at 15,000 rpm for 1 min in a microcentrifuge. A 200-µl aliquot of the supernatant was taken, added to 4 ml of scintillation fluid, and counted in a scintillation counter. The activity of the enzyme was calculated from the linear portion of the enzyme activity curve with respect to time and expressed as nmol substrate dephosphorylated min⁻¹ mg⁻¹ protein in the tissue extract.

Western blot analysis. Equal amounts of protein of tissue extracts (25 µg) were applied to 12.5% SDS-polyacrylamide gels and electrophoretically transferred to nitrocellulose paper. To facilitate the transfer of the proteins to the nitrocellulose paper, 0.1% SDS was included in the transfer buffer. We verified the efficiency of transfer by staining the gel with Coomassie blue after the transfer. The blot was blocked overnight with 5% nonfat dry milk in TTBS (20 mM Tris-HCl, pH 7.5, 500 mM NaCl, 0.05% Tween 20). The blots were incubated with the primary antibodies in TTBS with 1% nonfat milk and then with hors eradish peroxidase-conjugated secondary antibodies in TTBS containing 2.5% nonfat milk. All washes were carried out with TTBS containing 2.5% nonfat milk. The cross-reactivity of the antibodies was detected by chemiluminescence using a mixture of equal volumes of enhanced luminol reagent and oxidizing reagent. The following commercially available antibodies were used: clone K36 anti-MLCK monoclonal mouse antibody and monoclonal anti-calponin clone hCPC (Sigma, Oakville, Ontario, Canada), anti-PP1cδ (catalytic subunit of type 1 protein phosphatase MLCP) rabbit polyclonal antibody (Upstate, Lake Placid, NY), and anti-MYPT1 polyclonal (Santa Cruz Biotechnology, Santa Cruz, CA).

The cross-reactivity was quantitated by two-dimensional scanning of the autoradiograms of the blots with a high-resolution scanner (ImageMaster Analyser, Pharmacia). The content was quantitated from the optical density per band area (OD x mm²) and expressed as density unit/mg tissue extract protein.

Specific activity. The specific activities of MLCK and MLCP were calculated according to the following formula: enzyme activity (nmol min⁻¹ mg⁻¹)/enzyme content (density units/mg).

Organ bath studies. Third (fetal) and fourth-generation (newborn and adult) left lung intralobar pulmonary artery ring segments were dissected free and mounted in a wire myograph (Danish Myo Technology). The fetal, newborn, and adult muscular pulmonary artery ring segments used in the study had the following lumen diameters, respectively: 85.4 ± 0.4, 88.7 ± 0.9, 156 ± 9 µm. Isometric changes were digitized and recorded online (Myodaq, Danish Myo Technology). Tissues were bathed in Krebs-Henseleit buffer (in mM): 115 NaCl, 25 NaHCO₃, 1.38 Na₂HPO₄, 2.51 KCl, 2.46 MgSO₄·7 H₂O, 1.91 CaCl₂, and 5.56 dextrose, bubbled with air-6% CO₂, and maintained at 37°C.

After 1 h of equilibration, the optimal resting tension of the tissue was determined by repeated stimulation with 128 mM KCl until maximum active tension was reached. The optimal resting tensions differed between fetal, newborn, and adult tissues and were 1.07 ± 0.01, 1.63 ± 0.06, and 4.4 ± 0.2 mN, respectively. All subsequent force measurements were obtained at optimal resting tension.

Pulmonary vascular muscle force generation was evaluated by stimulating with KCl and the thromboxane A₂ mimetic U-46619.
Contractile responses were normalized to the tissue cross-sectional area as follows: \( \frac{\text{width}}{\text{diameter}} \times 2 \) and expressed as mN/mm². The relaxant response to sodium nitroprusside (SNP), 8-bromo-guanosine 3',5'-cyclic monophosphate (cGMP), and the Rho-kinase inhibitor Y-27632 was determined by precontracting the pulmonary arteries with U-46619 (10⁻⁶ M). This U-46619 concentration results in an average contraction of 75–80% of maximum (EC₇₅–₈₀) and is required to properly evaluate the fetal and newborn vessels given their lesser force development.

**Drugs.** All chemicals were obtained from Sigma Chemical (Oakville, ON, Canada) and dissolved in Krebs-Henseleit buffer.

**Statistical analysis.** Data were processed by one- or two-way analysis of variance, and the Tukey-Kramer test was used for multiple comparisons. All data are reported as means ± SE, and \( P < 0.05 \) was considered significant.

**RESULTS**

Determination of the mechanical properties of the pulmonary arteries showed significant age-related differences in the thromboxane A₂ analog U-46619 and KCl-induced force. In response to U-46619 at 10⁻⁷ and 10⁻⁶ M, the fetal and newborn pulmonary arteries generated a force equivalent to 20% of the adult values (Fig. 1). At the highest concentration of KCl tested, the adult arteries generated a >10-fold greater force compared with the fetal and newborn vessels (\( P < 0.01 \), Fig. 1). Similarly, in response to the nitric oxide donor sodium nitroprusside and cGMP, the U-46619-precontracted adult arteries relaxed to a significantly greater extent compared with the fetal and newborn. cGMP was only tested against the fetal vessels (\( P < 0.01 \), Fig. 2).

As a measure of the change of the pulmonary arterial smooth muscle protein content of the tissue extract, the calponin content was determined and is shown in Fig. 3. The calponin content increased with age and was significantly higher in the adult compared with the fetus and newborn (\( P < 0.01 \)).

The pulmonary arterial MLCK (Fig. 4A) and PP1cδ, the catalytic subunit of MLCP (Fig. 5A), content increased with age and were greatest in the adult (\( P < 0.01 \)). In contrast, determination of the enzyme activities per mg protein of tissue extracts showed no significant change with age (Figs. 4B and 5B). When calculated per enzyme density unit in 1 mg of protein of tissue extract (activity/enzyme content), the specific activities of MLCK and MLCP decreased with age (Figs. 4C and 5C) and were significantly (\( P < 0.01 \)) lower in the newborn and adult compared with the fetus.

To determine whether the maturational changes in MLCK and MLCP enzyme content and activity were unique to the pulmonary artery, we studied the aorta of animals of identical age. As shown in Fig. 6, the MLCK and MLCP content increased with age, but in contrast to the pulmonary arteries the adult aorta enzymes' specific activities were not significantly different than those of the fetus. When compared and solely due to slightly higher values in the newborn samples, the adult aorta MLCP-specific activity was statistically lower (\( P < 0.05 \)).

Purposely overexposed Western blotting analysis using K36 anti-MLCK monoclonal mouse antibody showed the presence...
of three isoforms of MLCK in fetal pulmonary artery extract (Fig. 7). Aside from the 130-kDa isoform of MLCK that was present in all tissue extracts analyzed (Figs. 4A and 6), higher-molecular-mass (200–220 kDa) isoforms were observed in the fetal and newborn, pulmonary arterial, but not in aorta, tissue.

Lastly, we sought to identify the factor(s) responsible for the maturational differences in MLCP activity in the pulmonary arterial tissue. We evaluated the pulmonary arterial MYPT content and Rho-kinase activation. The MYPT content significantly increased with age (P < 0.01) and was highest in the adult pulmonary arterial tissue (Fig. 8).

A significant maturational difference in relaxation to the Rho-kinase inhibitor Y-27632 was observed in U-46619-preconstricted vessels (Fig. 9). The Y-27632 compound is only a selective Rho-kinase inhibitor at molar concentrations <10⁻⁶ M and has a Ca²⁺-independent PKC isoenzyme inhibitory effect at higher concentrations (20, 25); whereas no response of adult arteries was observed with Y-27632 concentration lower than 10⁻⁵ M, the fetal and newborn vessels showed a statistically significant relaxation. Likely in keeping with the effect of the inhibitor on the PKC isoenzymes, complete relaxation was achieved for all vessels at a Y-27632 concentration of 10⁻⁴ M.

**DISCUSSION**

Maturational changes in the pulmonary and systemic vascular smooth muscle contraction and relaxation potential have been reported by us in sheep (6, 9) and by others in the rabbit (19, 26) and rat thoracic aorta (23). To the best of our knowledge this is the first developmental study of the rat pulmonary artery smooth muscle mechanical properties and its key cell enzymes responsible for contraction and relaxation. In this study we documented a significant age increase in the pulmonary arterial tissue smooth muscle potential for force development and relaxation. This was associated with a maturational increase in MLCK and MLCP content and a decrease in the enzyme-specific activities.

MLCK is a key regulatory enzyme responsible for smooth muscle contraction. Limited data, however, are available relative to its maturational expression in the vascular system. In the present study we documented that its pulmonary arterial, as well as aortal, content increases with age. Although no developmental data have been previously reported for the pulmonary artery in mammals, our findings are in keeping with the MLCK content of the sheep aorta where a significant increase was observed from fetal to adult life (3). Such an increase in the pulmonary and systemic vascular MLCK content likely ac-

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**Fig. 4.** Fetal (n = 6), newborn (n = 6), and adult (n = 6) pulmonary artery tissue extract myosin light chain kinase (MLCK) enzyme content (A), activity (B), and specific activity (C). PP1cδ, MLCP catalytic subunit. P < 0.01 compared with the fetal and newborn (***) and fetal (††) values by 1-way ANOVA and Tukey-Kramer multiple-comparisons test.

**Fig. 5.** Fetal (n = 6), newborn (n = 6), and adult (n = 6) pulmonary artery tissue extract myosin light chain phosphatase (MLCP) enzyme content (A), activity (B), and specific activity (C). PP1cδ, MLCP catalytic subunit. P < 0.01 compared with the fetal and newborn (**) and fetal (††) values by 1-way ANOVA and Tukey-Kramer multiple-comparisons test.
counts for the greater force observed in the adult when compared with the fetal vessels as documented in the pulmonary artery in this study and in the aorta by others (23). Yet a significant age difference in this enzyme-specific activity was noted for the pulmonary artery, but not aorta, indicating that prenatally a greater activity per unit mass is present in this tissue. Although this finding may appear to be at odds with the greater force development of the adult compared with the fetal pulmonary arteries, age difference in the vessels’ smooth muscle mass must be taken in account. As an indicator of smooth muscle mass the adult pulmonary arterial calponin content was about fourfold greater than the fetus, whereas the MLCK enzyme activity per unit of tissue extracted protein does not change with age. Thus developmentally regulated vascular muscle hyperplasia and increased MLCK content account for the greater force development potential of the adult pulmonary arteries.

Nevertheless, the higher fetal MLCK-specific activity of the pulmonary arterial smooth muscle is intriguing. It may possibly be related to maturational differences in its isoform distribution, since novel 200- to 220-kDa MLCK isoforms, which are distinct from smooth muscle and nonmuscle 130-kDa MLCK, have been identified (18). Expression of the 200- to 220-kDa isoforms is most abundant during early development, in contrast to the 130-kDa smooth muscle MLCK protein that is greatest in the adult (14, 16). The same finding was observed with the pulmonary artery MLCK in this study. Blue et al. (10)
have previously shown the presence of these high-molecular-mass isoforms in lung extract with this antibody. Consistent with the properties of the MLCK family, they phosphorylate the 20-kDa myosin light chains in a Ca\(^{2+}\)/calmodulin-dependent manner (10). But the activity of the higher-molecular-mass isoforms, compared with that of the 130 kDa in vascular smooth muscle, is presently unknown. Although the amount of the embryonic isoforms is very small compared with the 130-kDa isoenzyme, their existence raises the possibility that the higher MLCK-specific activity of the fetal and early neonatal pulmonary arterial smooth muscle may be partially attributed to the presence of the embryonic isoforms. Another plausible explanation for the higher MLCK-specific activity of the fetal pulmonary arteries is Rho-kinase stimulation. This is based on the evidence that Rho-kinase can increase MLCK-dependent myosin light chain phosphorylation (2) and Rho regulates MLCK activity in pulmonary arterial endothelial cells (17).

The other major finding of this study was the developmental changes in pulmonary arterial muscle relaxation and MLCP-specific activity. Smooth muscle relaxation has been shown to be dependent on the type 1 protein phosphatase MLCP, which is a trimeric enzyme composed of a 37-kDa catalytic subunit (PP1c), a 110-kDa regulatory subunit (MYPT), and a smaller 20-kDa subunit of unknown function (18). In the present study we showed that, whereas pulmonary arterial tissue PP1c and MYPT content increases with age, the specific activity of the enzyme is lowest in the adult. This finding appears to be unique to the pulmonary arterial tissue, since the adult rat aorta MLCP-specific activity was similar to the fetal vessels in the present study. As previously discussed for the pulmonary arterial MLCK, the lower MLCP-specific activity is not necessarily at odds with the greater relaxation potential observed in the adult, since the tissue enzyme content increases with age. Yet the reduced pulmonary vascular smooth muscle relaxant potential of the fetus and newborn shown in this study and previously reported by us in sheep (9) contrasts with the higher MLCP activity early in life and merits further discussion.

In the present study, we demonstrated a greater nitric oxide- and cGMP-mediated relaxation in the adult, compared with the fetal, rat pulmonary arterial muscle precontracted with the thromboxane A\(_2\) analog U-46619. In response to this agonist, the fetal and newborn intrapulmonary arterial muscle developed 20% of the force measured in adult rat arteries. This response to U-46619 is in keeping with measurements by Fuloria et al. (15) in the newborn pig pressurized pulmonary arteries, where a 30–45% constriction was observed at a 10\(^{-7}\) M concentration. Abman et al. (1) have previously reported no significant age difference in the relaxant response to SNP in sheep pulmonary arterial rings in vitro precontracted with phenylephrine. This discrepancy, however, may relate to the fact that relaxation is dependent on the agonist utilized to precontract the vascular preparation. In the newborn pigs Perez-Vizcaino et al. (28) have previously reported that noradrenaline-stimulated pulmonary arteries completely relaxed.
to SNP, whereas only partial relaxation was observed when these vessels were precontracted with U-46619. Further supporting the evidence that fetal vessels have a lesser relaxation potential, we have previously demonstrated that the load-independent, isometric, and isotonic half-relaxation time is significantly greater (reduced relaxation) in the fetal and newborn sheep compared with the adult values (7, 9).

Aside from a lower MLCP content, other factors may account for the lesser relaxation early in life. In the present study we documented that the fetal and newborn pulmonary arterial tissue has a lower MYPT content when compared with the adult. The rate of MLCP-induced relaxation has been shown to be dependent on the MYPT content and is lowest in the newborn mice bladder smooth muscle (13), suggesting that a similar mechanism may be operative in the pulmonary vascular tissue.

The induction of the GTPase RhoA promotes activation of the Rho-associated kinase, which phosphorylates the MYPT and inhibits MLCP-dependent relaxation (30). The hypoxia-induced RhoA expression in the lung is age dependent and found to be greatest in the fetus (4), yet developmentally dependent activation of this GTPase in the pulmonary vasculature has not been previously investigated. For this reason we evaluated the relaxant response to the Rho-kinase inhibitor Y-27632 in U-46619-precontracted rat pulmonary arteries and documented a significant maturational response difference. Compared with other agonists, the use of U-46619 to precontract the vessels has been shown by others to favor Rho-kinase activation (29). The evidence from this study that Rho-kinase inhibition with Y-27632 at low concentrations relaxes fetal and newborn, but not adult, vessels suggests that RhoA is expressed in greater amounts perinatally. This speculation is in keeping with the observation that Rho-kinase is important for lung morphogenesis during fetal life (24). Aside from the RhoA/Rho-kinase, inhibition of the MLCP has been reported to occur through CPI-17-dependent phosphorylation via PKCδ (31). This inhibitory pathway is independent of the Rho/Rho-kinase but is believed to only play a minor role in the G protein-coupled mechanism of Ca2⁺ sensitization (30). Given that we did not investigate the presence or potential development of regulation of CPI-17 in the pulmonary vasculature of the rat, we cannot exclude it as another possible explanation for the reduced relaxation of the fetus and newborn pulmonary arterial muscle.

Lastly, the physiological significance of the higher fetal MLCK- and MLCP-specific activity merits discussion. During fetal life the pulmonary vascular resistance is high as a result of its vascular smooth muscle contraction. Birth is associated with a >10-fold increase in pulmonary blood flow that occurs within minutes from the onset of breathing and is only possible because of rapid relaxation of the vascular smooth muscle. We speculate that effective relaxation of the pulmonary vascular muscle in the presence of activation of the RhoA/Rho-kinase during fetal life requires a higher MLCP-specific activity to offset the inhibitory effect of this pathway. Similarly the higher MLCK-specific activity, possibly the result of Rho-dependent activation, may contribute to the maintenance of a higher pulmonary vascular resistance in utero. Nevertheless, the physiological significance of the developmental changes in MLCK- and MLCP-specific activity warrants further studies to address their role in the control of pulmonary vascular resistance before and after birth.

In summary, we documented significant age-related changes in the pulmonary arterial muscle potential for contraction and relaxation, which can be explained on the basis of the maturational differences in tissue MLCK and MLCP content. The specific activities of both enzymes are highest in the fetal tissue and decrease with age. This novel finding appears to be related to Rho-kinase activation during lung morphogenesis and may contribute to the maintenance of a high pulmonary vascular resistance prenatally and the rapid relaxation immediately after birth.

GRANTS

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REFERENCES


