Myosin Isoform Shifts and Decreased Reactivity in Hypoxia-Induced Hypertensive Pulmonary Arterial Muscle

By: C. Subah Packer, Janet E. Roepke, Nicholas H. Oberlies, and Rodney A. Rhoades

"Myosin Isoform Shifts and Decreased Reactivity in Hypoxia-Induced Hypertensive Pulmonary Arterial Muscle." C. Subah Packer, Janet E. Roepke, Nicholas H. Oberlies, and Rodney A. Rhoades. *American Journal of Physiology-Lung Cellular and Molecular Physiology, 1998, 274,* L775-L785. PMID: 9612293.

Abstract:

The principal stimulus that evokes pulmonary hypertension is chronic alveolar hypoxia. Pulmonary hypertension is associated with remodeling of the vessel walls, involving hypertrophy and hyperplasia of pulmonary arterial smooth muscle (PASM) and a concomitant increase in the deposition of connective tissue, resulting in increased wall thickness. The purpose of the present study was to determine the effect of hypoxia-induced hypertension on the structure and function of PASM. Experiments were designed to determine whether hypoxia-induced pulmonary hypertension is associated with alterations in PASM: *1*) reactivity to a variety of agonists, *2*) contractile protein proportions and isoforms, and *3*) structural properties. Young adult male rats were made hypoxic by lowering the fraction of inspired O_2 (10%) for 14 days. Pulmonary arterial segments were isolated and dose-response curves to various agonists (high K^+, norepinephrine, serotonin, angiotensin II, and adenosine) were generated. Gel electrophoresis was used to measure changes in the relative amounts of actin or myosin and of myosin heavy chain (MHC) isoforms. Structural changes were correlated with the pharmacological and biochemical data. Hypoxia-induced pulmonary hypertension caused a general decreased reactivity, an increase in the proportion of nonmuscle to muscle MHC isoforms in PASM, and an increase in arterial wall thickness with PASM hypertrophy or hyperplasia.

Keywords: myosin heavy chain isoforms | arterial smooth muscle | decreased contractility | hypertrophy | hyperplasia

Article:

Pulmonary hypertension is a primary event leading to the development of right ventricular failure and respiratory failure. The principal stimulus that evokes pulmonary hypertension is chronic alveolar hypoxia. Local alveolar hypoxia causes an acute vasoconstriction and increased arterial resistance. When the hypoxia affects the whole lung rather than discrete regions and is

chronic rather than acute, pulmonary hypertension results. Remodeling of the vessel walls, including an increase in wall thickness by hypertrophy and hyperplasia of the pulmonary arterial smooth muscle (PASM) and an increase in the deposition of connective tissue, accompanies hypoxia-induced pulmonary hypertension (3, 21, 24, 30). Whether the vascular wall remodeling precedes or occurs concomitantly with the pulmonary hypertension has not been established. Reid (24) has suggested that although vasoconstriction plays a role in some types of hypertension, ultimately, the structural changes and not smooth muscle contractility per se are responsible for the luminal reduction and maintenance of high vascular pressures.

Griffith et al. (7) reported the results of an investigation of pulmonary arterial muscle contractility and pulmonary arterial wall mechanics that support Reid's (24) suggestion. Specifically, no increase in contractility was found in PASM from rats exposed to hypoxia for 14 days. In fact, although no change was found in the tension-velocity relationship, a decrease in the active stress-developing ability was actually found in the hypertensive muscle. However, increases in passive stiffness and wall thickness were found, supporting the idea that increases in wall thickness, and connective tissue in particular, contribute to hypoxia-induced pulmonary hypertension. Despite the fact that an increase in PASM contractility is not responsible for the maintenance of hypertension, the smooth muscle may still be the cell type primarily involved in the development and indirectly in the maintenance of hypoxia-induced pulmonary hypertension. Changes in smooth muscle cell function in response to hypoxia or high transmural pressure may be responsible for the vascular wall thickening and increased connective tissue. For instance, a smooth muscle phenotypic change from a contractile to a synthetic cell type could be responsible for the majority of structural changes in the vessel wall and maintenance of the high vascular resistance. A less contractile phenotype would be accompanied by either no change or a decrease in vascular smooth muscle reactivity. Such phenotypic changes are known to occur in other models of hypertrophic or hyperplastic vascular smooth muscle (1) and could explain the source of the increased connective tissue in the walls of the hypertensive vessels.

The purpose of the present study was to determine whether chronic hypoxia-induced pulmonary hypertension alters the sensitivity and/or reactivity of the PASM to a variety of agonists, including high K^+ and several physiological agonists {norepinephrine (NE), angiotensin II (ANG II), serotonin [5-hydroxytryptamine (5-HT)], and adenosine (Ado)}, and to determine whether myosin heavy chain (MHC) isoform shifts correlate with the decreased ability of PASM to develop active stress in hypoxia-induced pulmonary hypertension.

METHODS

Adult (10-wk-old) Sprague-Dawley rats weighing 300–324 g were made hypoxic by lowering the fraction of inspired O_2 (10%). Rats were placed in polycarbonate chambers, and N_2mixed with room air (Venturi system) was allowed to flow into the chambers at 1.5 l/min. The animals were made hypoxic by lowering the fraction of inspired O_2 to 15% for 24 h and then to 10% for the remainder of the 14 days. O_2 and CO_2 tensions were measured with Beckman O_2 (model C-2) and CO_2 (model LB-1) analyzers. O_2 tension was monitored continuously with a Beckman OM-15 O_2 meter. PO_2 was kept in the range of 75–80 Torr, and PCO_2 was kept in the range of 0.3–0.5 Torr. The chambers were opened every other day for feeding and cleaning to prevent

ammonia buildup. Control animals were maintained under similar conditions but were allowed to breathe room air.

After the 14-day hypoxic-exposure period, the rats were given an intraperitoneal injection of pentobarbital sodium (50 mg/kg body weight) and exsanguinated by section of the abdominal aorta. After the rats were anesthetized, blood samples for hematocrit determination were collected by insertion of a heparinized capillary tube into the area of the nasal canthus. Right ventricular pressure (P_{RV}), an index of pulmonary arterial pressure, was measured in the rats after 2 wk of chronic hypoxia and in age-matched control animals. To measure P_{RV}, a polyethylene catheter (inner diameter 23 μm, outer diameter 38 μm) was introduced into the right jugular vein and manipulated into the right ventricle. Pressure traces were displayed on a Hewlett-Packard patient monitor (model 78353B) that provided digital presentation of systolic, diastolic, and mean pressures. The heart and lungs were removed, and the hearts were dissected free of all atrial tissue. The ratio of the right ventricle to the left ventricle plus septum weights was determined and used as an index of the relative degree of right heart hypertrophy.

The lungs were placed in cold (4°C) Earle's balanced salt solution (EBSS; 2.4 mM $CaCl_2$, 0.8 mM $MgSO_4$, 5.4 mM KCl, 116.4 mM NaCl, 0.9 mM Na_2HPO_4, 5.5 mM d-glucose, 26.2 mM $NaHCO_3$ and 0.03 mM phenol red sodium). The two main branches of the pulmonary artery were excised and cleaned of all visible parenchyma and connective tissue under a dissecting microcope.

Some pulmonary arterial branches were prepared for morphometrics. In these cases, the pulmonary artery and its two major branches were isolated as described above except that the dissection was performed in Ca^{2+}-free, EGTA-containing Krebs-Henseleit buffer (to ensure that the smooth muscle was fully relaxed). Under the dissecting microscope, each branch was cut into two ring segments such that four ring segments were obtained from each rat. The arteries were held on end and kept patent with surgical steel posts while they were completely immersed in fixative (2.5% glutaraldehyde and 1.5% paraformaldehyde in 0.1 M cacodylate buffer at 4°C) for 2–4 h. Vessel segments were then postfixed in osmium tetroxide, dehydrated with alcohol, and embedded in Polybed 812. One-micrometer-thick sections were stained with toluidine blue.

Photographs were taken of each arterial segment through the ×2.5 objective of a Zeiss Ultraphot microscope. This magnification allowed visualization of the entire vessel cross section in one visual field. These whole section micrographs (final magnification ×45) were utilized to measure arterial cross-sectional area (CSA) and luminal diameter. All other light micrographs were taken through the ×10 objective, resulting in visualization of only a segment of the vessel wall at a time. These micrographs (final magnification ×208) were then arranged in a collage to recreate the entire vessel cross section and were used to measure wall thickness, medial thickness, adventitial thickness, and number of smooth muscle cell layers.

The ×45 micrographs were placed on a digitizing pad, and the inner and outer perimeters of each arterial wall were traced. With the use of planimetery software (SIGMASCAN, Jandel Scientific), two CSAs were calculated based on the inner and outer perimeters. The CSA of the vessel wall was then calculated by subtracting the inner CSA (i.e., lumen) from the outer CSA. A similar method was used to calculate the CSA of the medial layer.

The ×208 micrographs were arranged as a collage to reconstruct the images of the transverse arterial cross sections. These collages were analyzed with a drafter's T square to mark the vessel wall at points separated from each other by 90° angles. The T square was then rotated 45°, and four additional points of intersection were marked. At each of these eight points, the following measurements were made: total wall thickness, adventitial thickness, and number of smooth muscle cell layers. This process resulted in eight different independent measurements of each of these parameters for each vessel cross section. The eight measurements of each parameter were averaged for each section. Then the results, in mean values for each of the four sections of the same artery, were averaged together to give the mean wall thickness, medial thickness, adventitial thickness, and number of smooth muscle layers for each rat. The mean medial and adventitial thicknesses are expressed as percentages of the mean total wall thickness for each artery. Wall-to-lumen ratios were calculated from the wall thickness at each point divided by the mean luminal radius of the same section. Mean values from all the hypertensive arterial segments were then compared with those from control preparations.

For the reactivity studies, main pulmonary arterial rings ranging from 1.5 to 2.0 mm in diameter and from 2.5 to 3.5 mm in length were gently threaded onto horizontally oriented, fixed-position surgical steel rods (300 μm in diameter, 5 mm in length) located in the lower third of 20-ml volume glass muscle baths. Once anchored to this wire, a second wire of the same dimensions but suspended from a force transducer (Grass Instruments, Quincy, MA) that was connected to a chart recorder (Gould) was introduced into each lumen above the first wire. Each muscle bath contained 10 ml of EBSS aerated with 95% O_2-5% CO_2. The outer jacket of each muscle bath was connected to a circulating water bath maintained at 37°C.

In experiments in which ring segments denuded of endothelium were required, the endothelium was mechanically removed by threading the vessel onto a lightly sanded surgical steel rod and gently rotating the vessel twice in each direction. The integrity of the endothelium was tested by applying ACh (5×10^{-6} M) to NE-contracted arterial rings. Relaxation in response to ACh indicated insufficient removal of the endothelium, and data from such arterial rings were not used. In those experiments in which tachyphylaxis was a concern, this test of endothelial integrity was performed after washout of the last test drug used in the experiment.

Once mounted, the arterial rings were equilibrated for 1 h at a resting tension ranging from 5.5 to 8.0 mN. The optimal resting tension for maximal active tension development (P_o) was previously determined to be 7.0 ± 0.8 mN for normal rat pulmonary arterial segments (25). Because the rats used for that previous study were much older (400–500 g), this same optimal resting tension was verified in normal rat pulmonary arterial segments from rats of the same age (300–325 g) as those used for all experiments described in this study. In addition, a rather extensive preliminary study of the active versus resting tension relationship in hypoxia-induced hypertensve rat arterial rings was carried out. The hypertensive muscle has a broader resting versus active tension curve plateau region compared with the control muscle. Changing resting tension in the 7- to 16-mN range had no significant effect on active tension production in the hypertensive preparations. These findings are similar to those reported by Griffith et al. (7). Therefore, an optimal resting tension value of 7.0 mN was used as the baseline tension for all experiments unless otherwise

specified. Equilibration was followed by a maximal contraction with 80 mM KCl. The peak tension developed (in mN) was normalized to the CSA of the tissue (in mN/mm^2).

At the end of the pharmacological experiments, the length and width (diameter) of each vessel segment were measured. The vessel segments were then blotted, and the wet weight was obtained. Because the density of the tissue is close to one (1 mg/1 mm^3), the weight of the tissue in milligrams is an estimate of the volume of tissue in cubic millimeters (i.e., density = mass/volume). The volume divided by the width of the muscle is then approximately equal to the CSA across which tension is developed (17). P_o was determined for each vessel segment used in the pharmacological experiments and was defined as the maximum tension developed in response to supramaximal stimulation with 80 mM KCl. As the amount of muscle increases with thicker or longer ring segments, the absolute tension developed is also greater. Therefore, P_o must be normalized to CSA for comparative purposes: P_o/CSA = tension (in mN) developed in response to 80 mM KCl/[volume (in mm^3)/width (in mm)].

The high-K^+ solution used to obtain P_o was subsequently washed out, and the muscle was allowed to relax completely. Each ring was then exposed to an agonist (5-HT, NE, ANG II, or Ado) with different randomly applied or cumulative doses (depending on the agonist). Example tension versus time tracings are shown in Fig.1. The magnitude of each response was normalized by expressing it as a percentage of the response to 80 mM KCl (%P_o).

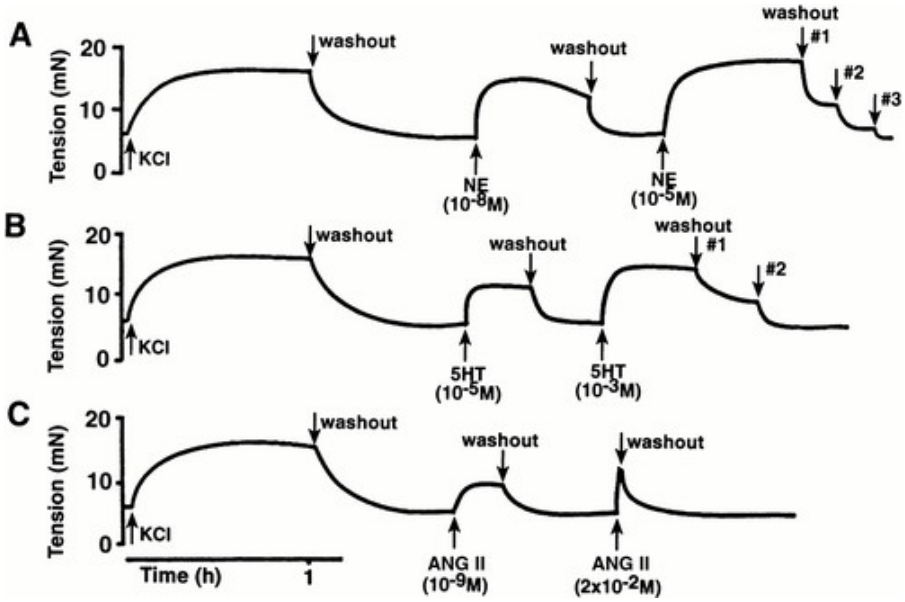

Fig. 1. Tension vs. time tracings. An 80 mM KCl control contraction was followed by washout. Then a norepinephrine (NE;*A*)-, serotonin [5-hydroxytryptamine (5-HT)*B*]-, or angiotensin II (ANG II;*C*)-induced contraction was allowed to peak, and bath was flushed again. After complete relaxation, a higher dose of NE, 5-HT, or ANG II was introduced into the bath, resulting in a greater contractile response. Once the contractile response had reached a peak, vessel bath was again washed out, and relaxation was achieved. Tension produced in response to several different doses of NE, 5-HT, and ANG II was measured from this type of data, and complete dose-response curves were generated. Nos. in parentheses, dose.

Each agonist solution was prepared as a high-concentration "stock" solution, divided into 1- to 2-ml aliquots, and then frozen at −70°C. Serial dilutions of each stock solution were made on the day of the experiment, providing a series of agonist solutions that covered a range of several

orders of magnitude (i.e., 10^{-10} to 10^{-3} M). For agonists that showed no tachyphylaxis (NE and Ado), it was possible to give additive doses to a given vessel without washing between doses. Serial-dilution solutions were added to the smooth muscle baths in small aliquots such that after each dose was added, the final concentration of the agonist was 10 times higher than the previous dose. For the other agonists (5-HT and ANG II), a single dose-response protocol (i.e., washing between doses) was utilized to ensure that tachyphylaxis of the muscle did not cause error in the dose-response curves generated.

NE stock solution was made by dissolving 42.25 g of NE HCl (mol wt 205.6; Arterenol, Sigma) in 50 ml of sterile 0.9% NaCl. One hour after washout of the high-K^+ solution, when tension reached a steady baseline, aliquots of this solution were added to the tissue bath in the following order of cumulative final doses: 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, and 5×10^{-5} M.

ANG II stock solution was made by dissolving 5 mg of ANG II (human synthetic form acetate salt, mol wt 1,046.3; Sigma) in 5 ml of sterile H_2O. One hour after washout of the high-K^+solution, when tension reached a steady baseline, a single dose of ANG II was added to each bath. Because ANG II was one of the agonists that resulted in tachyphylaxis, a cumulative dose-response protocol was not used. For the single dose-response experiments, aliquots of ANG II were added to the tissue bath to provide one of the following final concentrations of ANG II for each arterial ring: 10^{-6}, 10^{-7}, 2×10^{-8}, 10^{-9}, 10^{-10}, and 10^{-11} M.

5-HT stock solution was made by dissolving 0.1937 g of 5-HT (creatinine sulfate complex, mol wt 387.4; Sigma) in 3 ml of sterile H_2O + 1 ml of EBSS. Then 0.100 ml of 5 N NaOH was added to bring the solution to a pH close to 7.4 (i.e., within the pH range of the phenol red sodium indicator that is a component of EBSS). One hour after washout of the high-K^+solution, when tension reached a steady baseline, aliquots of these solutions were added to the tissue bath to create one of the following final concentrations of 5-HT in the tissue bath: 10^{-7}, 10^{-6}, 10^{-5}, 2×10^{-5}, 10^{-4}, or 10^{-3} M.

Ado stock solution was made by dissolving 116.3 mg of Ado (hemisulfate form, mol wt 316.3; Sigma) in 10 ml of sterile H_2O. One hour after washout of the high-K^+ solution, when tension reached a steady baseline, vessels were precontracted with 10^{-5} M NE. As soon as the response to NE reached a plateau, aliquots of the Ado solution were added to the tissue bath in a sequential manner to reach cumulative concentrations of Ado as follows: 10^{-8}, 10^{-7}, 10^{-6}, 10^{-4}, and 10^{-3} M.

KCl stock solution was made by dissolving 18.64 g of KCl (mol wt 58.44; Sigma) in 100 ml of sterile H_2O and was stored at 4°C until used. For the initial maximal contraction in each pharmacological experiment, 320 μl of the KCl solution were added to each bath to give a final concentration of 80 mM KCl, previously shown to be more than sufficient to ensure a maximum isometric contraction (25).

To determine whether hypoxia-induced pulmonary hypertension or removal of the endothelium resulted in changes in PASM reactivity or sensitivity to various physiological agents, dose-response curves of the vasoconstrictors NE, ANG II, and 5-HT and to the vasodilator Ado were generated and compared.

In other experiments, the main pulmonary arterial rings were prepared for MHC isoform and actin quantification. The main pulmonary arteries were dissected as described for the isometric tension studies. Due to the small absolute amount of muscle in rat pulmonary arteries, the two main branches and the trunk were used, and the muscle tissue of pulmonary arteries from four to five rats was combined for each sample. The arterial tissue was frozen in liquid N_2, pulverized, acetone dried, desiccated under a low vacuum, and then stored at −70°C until used. For each experiment, 0.6 mg of each sample was dissolved in 100 μl of SDS-gel dissociation medium. The mixture was heated at 100°C for 30 min and centrifuged, and the supernatant was then applied to 5% acrylamide-0.75%*N*,*N*′-methylene-bis-acrylamide (BIS; 1.50% BIS for actin) gels with the buffer system of Porzio and Pearson (23). The samples were subjected to electrophoresis along with heavy-molecular-weight and BSA standards at 300 V and constant voltage at 10°C for ~2–3 h for separation of actin and myosin and for 5 h for MHC isoform separation. After electrophoresis, the gels were stained with Coomassie blue, and myosin content was determined by quantitative densitometric scanning.

Student's *t*-test was used when comparing any two mean values for the hypertensive and control data. All results are expressed as mean values ± SE, and$P < 0.05$ is indicative of mean values that are significantly different from one another. Differences between any two cumulative dose-response curves were demonstrated with a multiple (repeated)-measures ANOVA. For dose-response curves obtained from individual (rather than cumulative) dose-response experiments, a two-way ANOVA for independent measures was utilized. To compare any two mean values at a given dose, Student's*t*-test was used. Myosin isoform ratios were compared with one-way ANOVA followed by the Newman-Keuls test.

RESULTS

The mean hematocrit for hypoxic rats (57.0 ± 1.0%; $n = 9$) is significantly greater than that of age-matched control rats (42.0 ± 1.5%; $n = 13$; $P < 10^{-5}$). The right ventricle-to-left ventricle plus septum weight ratio for hypoxic rats ($n = 24$) is also significantly greater than that for control rats (0.40 ± 0.01 and 0.25 ± 0.01, respectively; $n = 18$; $P < 10^{-6}$). The mean hypoxic rat ($n = 4$) P_{RV} of 16.0 ± 0.7 mmHg is significantly higher than the mean control rat ($n = 6$) P_{RV} of 9.5 ± 0.3 mmHg ($P < 0.00005$).

Both hypertensive ($n = 4$) and control ($n = 4$) pulmonary arteries demonstrated a loose outer adventitial connective tissue layer and inner organized smooth muscle layers. But only the hypertensive arteries had a poorly organized layer of cells between the inner organized smooth muscle layers and the outer connective tissue layer. Enlarged views of histological sections (×208) of arteries are shown in Fig. 2. The increase in the number of smooth muscle cell layers and the presence of an amorphous layer with either nonmuscle or migrated smooth muscle cells in the hypertensive arteries are evident in these micrographs. Figure2*B*shows palisading nuclei within the inner, more organized smooth muscle cell layers, suggesting that the smooth muscle cells were cut in cross section and lie parallel to the longitudinal axis of the vessels. The amorphous layer does not show such palisading nuclei, and the direction in which these cells are oriented could not be determined. None of the sections demonstrates any clear changes in the

intima associated with hypoxia-induced hypertension. There was no evidence of intimal proliferation, and no atherosclerotic changes were seen with light microscopy.

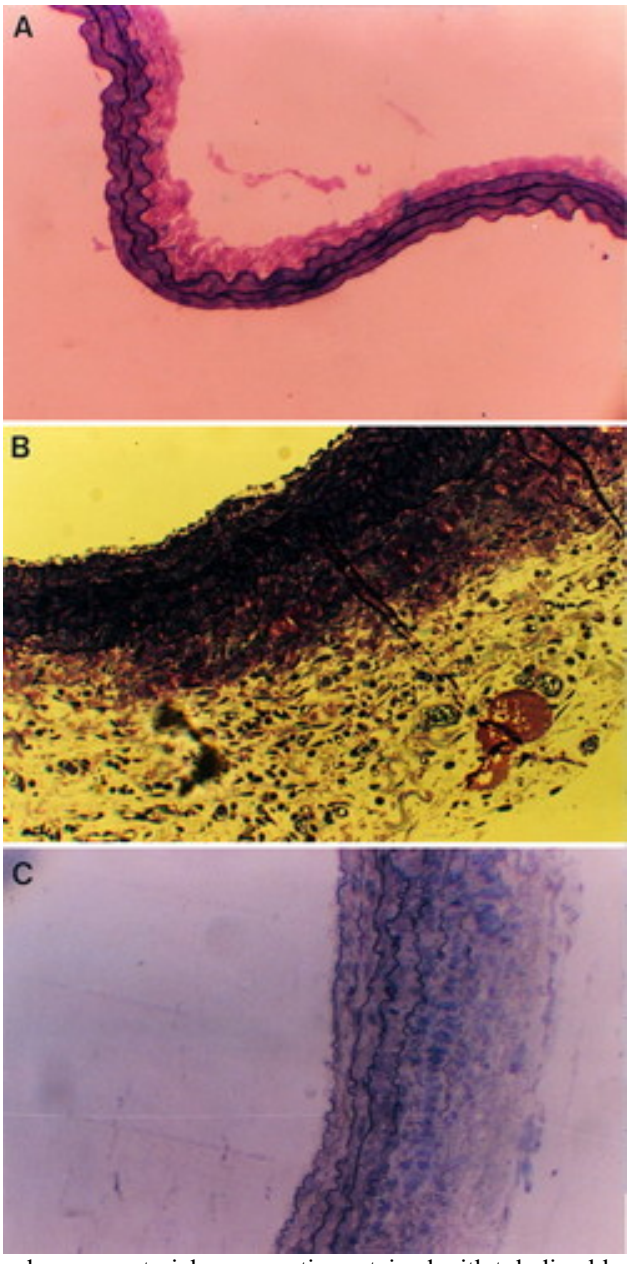

Fig. 2. Micrographs of main pulmonary arterial cross sections stained with toludine blue. Hypertensive arteries (*B* and *C*) have much thicker walls, increased number of smooth muscle layers and an additional amorphous layer of cells compared with control artery (*A*). Also note presence of palisading nuclei in organized smooth muscle layers, indicating that cells are parallel to one another and to longitudinal axis of vessel wall (*B*) but are lacking in the amorphous layer (*C*) of hypertensive arterial section, indicative of disorganization, possibly due to hyperplasia rather than to hypertrophy. Final magnification, ×208.

The mean CSA for control and hypertensive pulmonary arteries and other morphometric values determined from light micrographs are shown in Table 1. The mean CSA of hypertensive pulmonary arteries measured from light micrographs is approximately double the mean CSA of control pulmonary arteries. The mean hypertensive pulmonary arterial wall thickness and

adventitial and medial thicknesses are all significantly greater than the mean control thicknesses. Even when the medial thickness of the hypertensive pulmonary arteries was measured without including the outer amorphous muscle layer (media − outer layer), the mean is significantly greater than that of the control arteries. The mean number of smooth muscle cell layers is increased, whereas the thickness of the cell layers, excluding the outer amorphous layer, is unchanged in the hypertensive pulmonary arteries relative to the control arteries. The mean luminal radius is also not different between the hypertensive and control arteries. Although there is a significant increase in the mean adventitial thickness in the hypertensive pulmonary arteries, the proportion (in percent) of the wall that is composed of adventitia in the hypertensive arteries is not significantly different from that in the control arteries. Similarly, the proportion of the medial thickness relative to the wall thickness is unchanged in the hypertensive arteries relative to that in the control arteries. Although the radius of the lumen is not different, the wall thickness is increased in the hypertensive pulmonary arteries as reported above, and, therefore, the wall-to-lumen ratio in the hypertensive arteries is significantly greater than that in the control arteries.

Table 1. Rat pulmonary artery morphometrics

Parameter	Hypertensive	Control
Wall thickness, mm	0.152 ± 0.006‡	0.076 ± 0.005
Media, mm	0.105 ± 0.003‡	0.047 ± 0.003
% Wall	69.2 ± 3.3*	61.8 ± 2.1
Media − OL, mm	0.059 ± 0.001*	0.047 ± 0.003
% Wall	39.1 ± 0.8‡	61.8 ± 2.1
Adventitia, mm	0.047 ± 0.006*	0.029 ± 0.003
% Wall	30.8 ± 3.3	38.3 ± 2.0
Luminal radius, mm	0.755 ± 0.009	0.763 ± 0.019
Wall-to-lumen ratio, %	20.1 ± 0.7‡	9.9 ± 0.7
No. of smooth muscle layers	5.2 ± 0.4†	3.1 ± 0.1
Individual smooth muscle cell layer thickness, mm	0.014 ± 0.001	0.016 ± 0.001
Wall cross-sectional area, mm^2	0.92 ± 0.11‡	0.44 ± 0.03
Media cross-sectional area, mm^2	0.49 ± 0.03‡	0.27 ± 0.02

Values are means ± SE; $n = 4$ arteries/group. OL, outer amorphous layer of smooth muscle cells. Significantly different from control arteries: *$P < 0.05$; †$P < 0.005$; ‡$P < 0.001$.

Control vessels with and without endothelium responded to doses of NE $\geq 10^{-6}$ M with sustained contractions for >1 h. Hypertensive vessels ($n = 49$) required a dose of $\geq 10^{-5}$ M NE to evoke a sustained response. The NE dose-response curves for pulmonary arterial preparations with and without endothelium are shown in Fig.3*A*. There is a general upward shift in the dose-response curve for pulmonary arteries without endothelium ($F = 23.987$; $P < 0.001$). The NE dose-response curves for hypertensive and control pulmonary arteries obtained from single-dose experiments are shown in Fig.3*B*. There is a general downward shift in the hypertensive dose-response curve relative to the control curve ($F = 116.878$; $P < 0.001$). The mean maximum response of the hypertensive arteries (95.9 ± 8.4%P_o; $n = 9$) is significantly lower than the mean control response to the same dose (133 ± 0.2%P_o; $n = 6$; $P < 0.05$). The NE dose-response curves for control pulmonary arteries (with endothelium) exposed to the same range of NE doses as those used above but under acute normoxic and hypoxic conditions are shown in Fig.3*C*. The PO_2 measured in the hypoxic tissue baths was 28.3 ± 1.2 Torr ($n = 4$ arteries). Acute in vitro

hypoxia resulted in a general downward shift in the NE dose-response curve relative to the control curve (F= 140.839; $P < 0.001$).

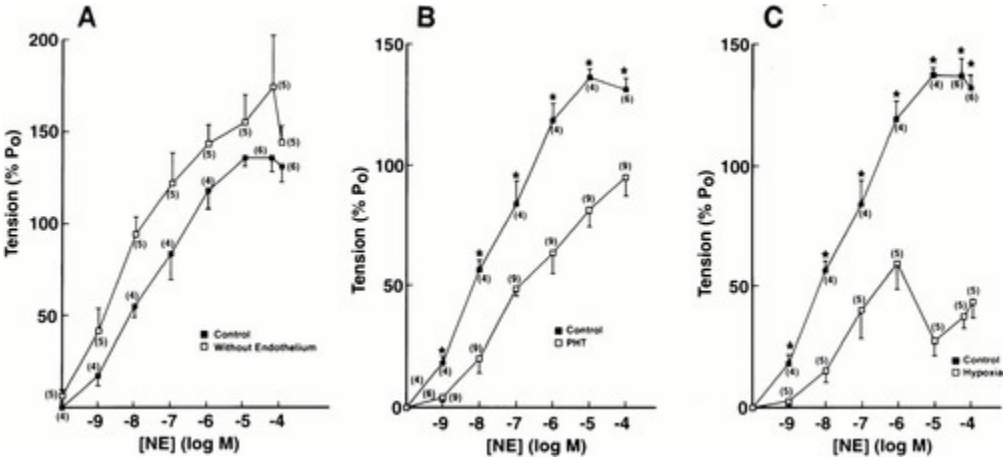

Fig. 3. NE dose-response curves for endothelium-denuded pulmonary arterial muscle (*A*), hypoxia-induced pulmonary hypertensive (PHT) pulmonary arterial muscle (*B*), and control pulmonary arterial muscle under in vitro normoxic and acute hypoxic conditions (*C*). $\%P_o$, maximal active tension development normalized by expression as percentage of response to 80 mM KCl; [NE], NE concentration. Nos. in parentheses, no. of arteries. PHT curve was shifted below control curve, indicating decreased reactivity of PHT arterial muscle to NE. Curve for muscle under hypoxic conditions was shifted well below control curve. * $P < 0.05$.

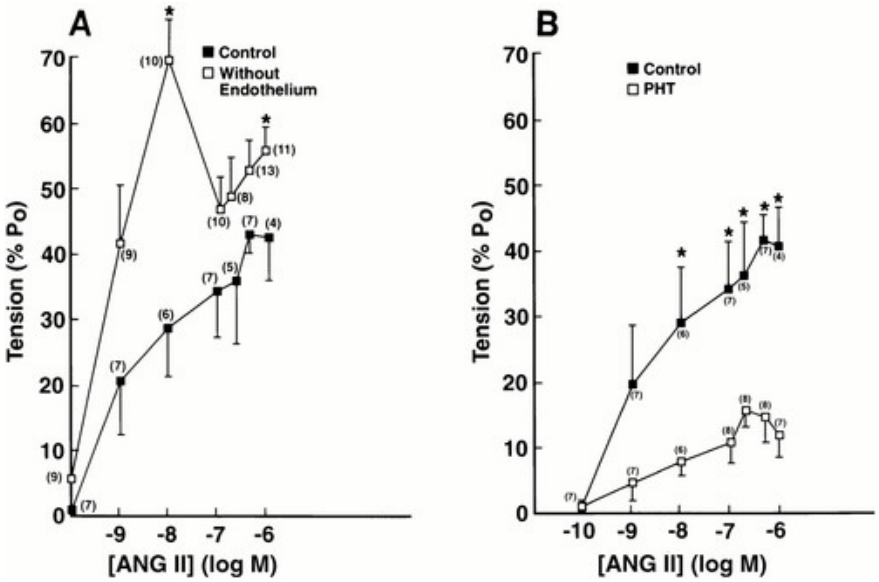

Fig. 4. ANG II dose-response curves for endothelium-denuded (*A*) and PHT (*B*) rat pulmonary arterial muscle compared with control muscle. [ANG II], ANG II concentration. Nos. in parentheses, no. of arteries. Endothelium-denuded muscle was more reactive than endothelium-intact muscle. PHT curve was shifted well below control curve, indicating decreased reactivity of PHT arterial muscle to ANG II. *$P < 0.05$.

The ANG II dose-response curves for pulmonary arteries with and without endothelium are shown in Fig.4*A*. There is a general upward shift in the dose-response curve for denuded vessels compared with control arteries (F = 23.987; $P < 0.001$). The mean maximum response of the arteries without endothelium (70.2 ± 6.8%P_o; n = 10) occurred at 10^{-8} M ANG II and is significantly greater than that of control arteries with endothelium (29.6 ± 8.1%P_o; n = 6; $P <$

0.05) at the same dose. The ANG II dose-response curves for hypertensive and control pulmonary arteries (both with endothelium) are shown in Fig.4*B*. There is a general downward shift in the hypertensive dose-response curve relative to the control curve, as in the case of NE ($F = 48.024$; $P < 0.001$). The mean maximum response of the hypertensive arteries to ANG II ($17.3 \pm 3.0\%P_o$; $n = 8$) is significantly different from that of the control arteries at the same dose ($36.2 \pm 9.3\%P_o$; $n = 5$; $P < 0.05$).

The mean dose-response curves for 5-HT in pulmonary arteries with and without endothelium are shown in Fig.5*A*. There is a general upward shift in the dose-response curve for vessels without endothelium ($F = 47.34$; $P < 0.001$). The mean maximum response to 5-HT is $95.6 \pm 2.4\%P_o$ in the segments without endothelium ($n = 3$), which is significantly greater than the mean maximum response for control segments ($46.3 \pm 5.8\%P_o$;$n = 6$;$P < 0.05$). The 5-HT dose-response curves for hypertensive and control pulmonary arteries (both with endothelium) are shown in Fig. 5*B*. There is no significant difference between the hypertensive and control dose-response curves for 5-HT ($F = 3.33$; $P > 0.05$). The mean maximum response of the hypertensive arteries to 5-HT ($61.4 \pm 8.9\%P_o$; $n = 3$) is not significantly different from the mean control response at the same dose ($53.0 \pm 9.4\%P_o$; $n = 6$; $P > 0.05$).

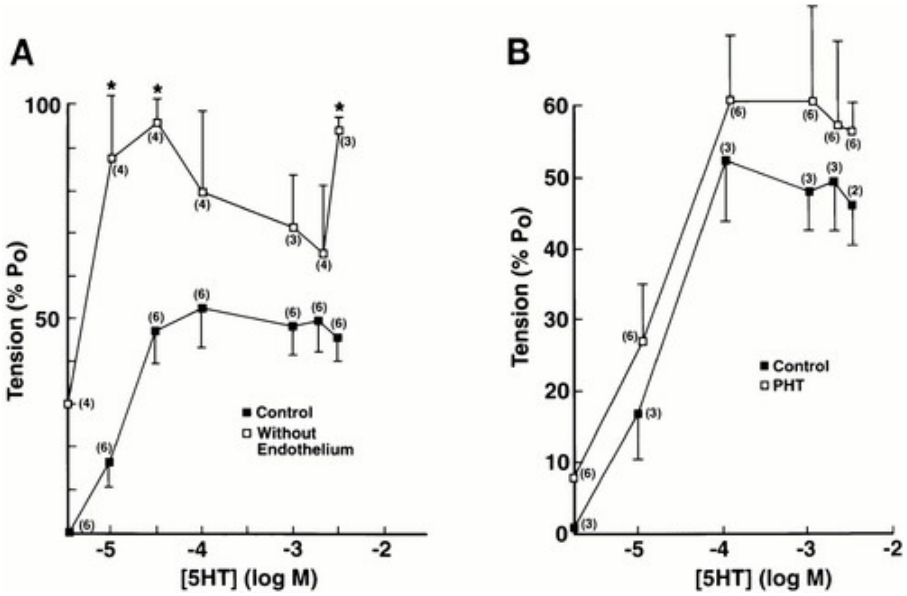

Fig. 5. 5-HT dose-response curves for endothelium-denuded (*A*) and PHT (*B*) rat pulmonary arterial muscle compared with control muscle. [5-HT], 5-HT concentration. Nos. in parentheses, no. of arteries. Disruption of endothelium results in increased reactivity to 5-HT as for NE and ANG II. Control and PHT curves are not different, $P > 0.05$; *$P < 0.001$.

No response to Ado could be elicited in vessels unless they were actively precontracted. Arterial segments ($n = 4$) at resting tension did not respond to any dose of Ado. NE was chosen as the agonist for precontracting the vessels because it provided a reproducible response to a given dose and a relatively sustained plateau (i.e., >1 h for doses > 10^{-6} M; $n = 21$). Doses of 10^{-8} to 10^{-3} M Ado did not elicit any response in the resting vessels but resulted in either contraction or relaxation of NE-precontracted pulmonary arteries depending on the Ado concentration as previously reported (26). At low doses of Ado, the response varied from slight relaxation to slight contraction. At higher doses of Ado, only relaxation occurred. The response to a given

dose of Ado was usually maintained regardless of whether it resulted in an increase or decrease in tension. Only control vessels displayed contraction in response to low doses of Ado.

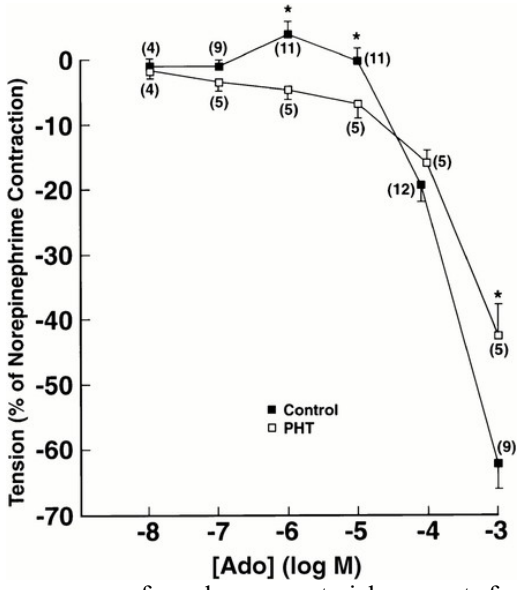

Fig. 6. Adenosine (Ado) dose-response curves for pulmonary arterial segments from control and hypoxia-induced PHT rats. Ado responses are expressed as percent of response to 10^{-5} M NE. [Ado], Ado concentration. Nos. in parentheses, no. of arteries. The 2 curves are similar except for small contractile response of control pulmonary arterial smooth muscle at low doses (10^{-6} and 10^{-5} M) of Ado and decreased relaxation response of PHT muscle at a higher dose (10^{-3} M) of Ado. * $P < 0.05$.

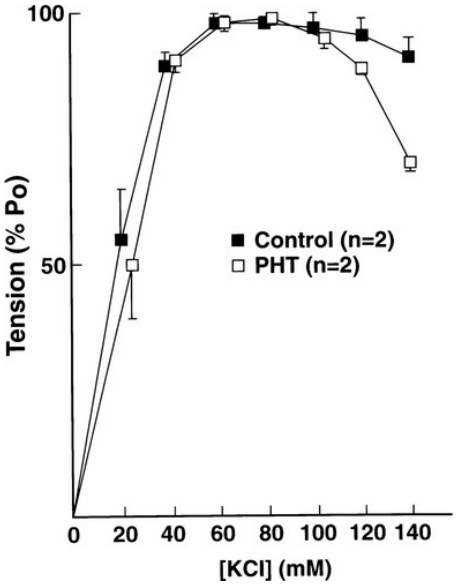

Fig. 7. KCl dose-response curves for rat pulmonary arterial smooth muscle. [KCl], KCl concentration. *n*, No. of arteries. Comparison of mean control and hypoxia-induced PHT curves shows no difference, $P > 0.05$. However, this method of tension normalization (i.e., $\%P_o$) does not take changes in wall content of muscle or in muscle function into consideration.

The effect of endothelium removal on the response of pulmonary arterial muscle to Ado has been previously reported (26). Briefly, there was a slight contractile response at 10^{-6} M Ado in vessels with intact endothelium ($4.1 \pm 2.2\%P_o$;$n = 10$;$P < 0.05$). No contractile response to Ado was

observed for rat pulmonary arteries without endothelium. Vessels denuded of endothelium responded with relaxation over the range of 10^{-7} to 10^{-3} M Ado. Higher doses of Ado ($\geq 10^{-4}$ M) resulted in relaxation of the same magnitude in both vessels with intact endothelium and vessels without endothelium. The mean Ado dose-response curves for hypertensive and control pulmonary arteries are shown in Fig. 6. Unlike control arteries, no contractile response to Ado was observed for hypertensive rat pulmonary arteries. The hypertensive pulmonary arteries responded to Ado with relaxation over the dose range of 10^{-8} to 10^{-3} M. At the highest dose of Ado (10^{-3} M), the hypertensive arteries did not demonstrate as much relaxation ($-42.2 \pm 4.9\%$; $n = 5$) as the control arteries.

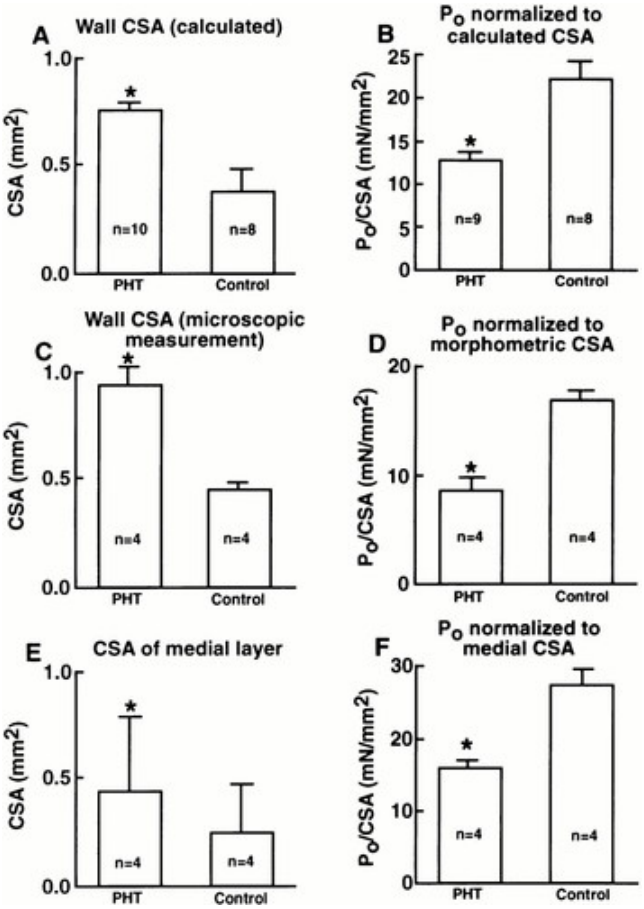

Fig. 8. Comparison of cross-sectional area (CSA;*A*, *C*, and *E*) and P_o normalized to CSA (*B*, *D*, and *F*) for control pulmonary arterial segments with segments from hypoxia-induced PHT rats using 2 different methods of determination of CSA. *n*, No. of arteries. Total calculated CSA of hypertensive pulmonary arterial segments is greater than that of control segments (*A*). P_o normalized to calculated CSA is less in hypertensive than in control pulmonary arterial muscle (*B*). These conclusions hold true when CSA is determined by morphometric analyses (*C* and *D*, respectively) and also when only vessel wall medial layer CSA is compared (*E*) and when P_o is normalized to medial CSA rather than to total (i.e., whole wall) CSA (*F*). * $P < 0.05$ compared with respective control arteries.

The KCl dose-response curves from hypertensive and control pulmonary arteries are shown in Fig. 7. Arterial ring segments were exposed to KCl doses in the range of 20–120 mM, and the active responses are expressed as a percentage of the maximal response to 80 mM KCl. This method of normalization of the responses allows for comparison of the mean dose-response

curves to ensure that when the rats were made hypertensive, the sensitivity and relative responsiveness to KCl were not altered. The curves are almost superimposable, and there is no significant difference in the response at any dose of KCl ($P > 0.05$). Tension-generating ability was determined by comparing responses normalized to the amount of smooth muscle (i.e., CSA) as shown in Fig.8. The hypertensive pulmonary arterial muscle tension-generating ability is reduced compared with control preparations when normalized for the proportionate amount of muscle in the tissue CSA ($P < 0.05$).

Analyses of densitometric scans of the 1.5% BIS-SDS-polyacrylamide gels showed that there was no difference in the amount of actin relative to the amount of total protein (i.e., peak areas/mg dry weight of total protein) in hypertensive compared with control myofibrillar protein (1,100 ± 120 and 988 ± 110 mm^2/mg protein, respectively). Similarly, there is no difference in the actin-to-myosin ratios of hypertensive PASM (2.19 ± 0.12) and the control PASM (1.90 ± 0.14). Scans of the 0.75% BIS-SDS-polyacrylamide gels revealed four distinct myosin isoforms for both control and hypertensive pulmonary arterial muscles (20). The four MHCs have molecular masses of ~204, 200, 196–198, and 190 kDa (MHC_{204}, MHC_{200}, MHC_{196}, and MHC_{190}, respectively). Other investigators have identified four isoforms in other smooth muscles (5) and the latter two isoforms in nonmuscle cells (16). The relative amounts of these various isoforms in hypertensive and control pulmonary arterial muscles are compared in Table 2. The hypertensive and control ratios of MHC_{200} to MHC_{204} are not significantly different ($P > 0.05$). However, the $MHC_{190+196}/MHC_{200+204}$ratio is higher in the hypertensive than in the control pulmonary arterial muscle ($P < 0.05$).

Table 2. Comparison of pulmonary arterial MHC isoform ratios

	Hypertensive	**Control**
MHC_{200}/MHC_{204}	0.987 ± 0.417	1.134 ± 0.229
	(n = 4)	(n = 8)
$MHC_{196+190}/MHC_{204+200}$	0.339 ± 0.041†	0.136 ± 0.068
	(n = 4)	(n = 4)
$MHC_{196}/MHC_{204+200}$	0.256 ± 0.032*	0.178 ± 0.032
	(n = 8)	(n = 8)

Values are means ± SE; n, no. of electrophoresis runs. Value from each run is actually a mean value of 4 lanes (4 samples). And, in turn, each sample represents protein from pulmonary arteries of 5 rats. Therefore, each n represents data from 20 or more rats. MHC, myosin heavy chain. Subscript nos., molecular mass (in kDa) of MHC isoforms. Significantly different from control values: $^{*}P < 0.02$; $^{\dagger}P < 0.00002$.

DISCUSSION

Results of this study show that after 14 days of hypoxia there is an increase in the adventitial and medial thicknesses but no change in the percent contribution of the media to the wall thickness in rat pulmonary arterial walls. Similar results have been previously reported (7). Reid (24) found approximately a doubling in thickness of the arterial smooth muscle in all the muscular arteries as well as an extension of muscle into previously nonmuscular peripheral arteries. This doubling of muscle means a greater absolute amount of muscle. An increase in the mean number of layers of smooth muscle from 3.1 in control arteries to 5.2 in hypertensive pulmonary arteries, with no increase in the thickness of each muscle layer, as shown in this study, suggests smooth muscle hyperplasia has occurred at this stage (14 days of hypoxia). The luminal radius is not altered in

the hypertensive arteries, but the adventitial layer is almost double that observed for control arteries. In addition, there is an increase in the number of cells in the adventitial layer close to the media. These cells are not organized into a layer with uniform thickness like the medial layers nor are the elastic fibers organized into a continuous concentric band, suggesting that either hyperplasia of smooth muscle is not occurring on the luminal side of the vessel but rather on the adventitial side, at least in the large hilar arteries utilized in this study, or nonmuscle cells such as fibroblasts are proliferating in the adventitia of hypertensive arteries.

A response to a given agonist depends on the number of receptors, affinity of the receptors, intracellular coupling mechanisms, and, ultimately, the number of tension-generating sites (i.e., actin and myosin interactions) in vascular smooth muscle. Normalizing the P_o produced to the tissue CSA provides an index of the number of tension-generating sites per unit of wall CSA. However, this method of normalization does not take into consideration changes in vessel wall composition. A method of normalization that allows for comparisons of changes in reactivity to the specific agonist regardless of changes in wall structure is to express the response as a proportion of a maximal contraction for the preparation in response to membrane depolarization ($\%P_o$). Normalization of agonist responses to the magnitude of the maximal KCl response allows for determination of whether differences in the active responses to various agonists are specific to a particular agonist or are due to a general alteration in contractility.

The pulmonary arterial segments from both control and hypertensive rats responded to the same range of NE doses (i.e., 10^{-10} to 10^{-4} M), with a peak response at 10^{-5} to 10^{-4} M, in agreement with that reported by other investigators (13). The general upward shift in the NE dose-response curve for endothelium-denuded vessels is likely simply due to a decrease in endothelium-derived relaxing factor because there is increased reactivity of the PASM to all contractile agonists (ANG II, NE, and 5-HT) investigated when the endothelium is rendered nonfunctional. The fact that sustained tension in the hypertensive vessels required a higher dose ($\geq 10^{-5}$ M) than in control vessels suggests that there may be a change in receptor function or a coupling when the smooth muscle is made chronically hypoxic. β-Adrenergic receptors could play a greater role in hypertensive compared with control rats, and, therefore, a higher dose of NE would be required to overcome the relaxation effects of the β-receptors. This idea is supported by the fact that, after 5 days to 2 wk of hypoxia, pulmonary arteries show less vasoconstriction in response to NE (12, 19, 22) and that the vasoconstriction is augmented with β-receptor blockade (12, 22). A decrease in ability to develop tension relative to the CSA could be due to the loss of contractile function in some of the smooth muscle cells and/or a disproportionate increase in connective tissue. However, the NE responses were normalized to P_o, and, therefore, the decrease in the absolute ability of the smooth muscle to develop tension cannot account for the decrease in NE reactivity observed. Therefore, a change in NE-receptor function, such as receptor downregulation, must have occurred with the development of pulmonary hypertension. A decreased vasoconstriction (decreased vascular resistance) in response to NE has also been reported in isolated lungs from chronically hypoxic rats (22).

A decrease in reactivity to NE was also observed in isolated pulmonary arteries from control rats that were exposed to acute hypoxia (i.e., in vitro hypoxia). This is possibly due to a generalized decrease in reactivity in response to all agonists during acute hypoxia because Lloyd (11) showed that hypoxic media decreased the response to electrical stimulation, 5-HT, NE, ANG,

K^+, and ACh without altering resting tension. Harabin et al. (8) found a decrease in reactivity to $PGF_{2\alpha}$ and ANG II in isolated perfused pig lungs exposed to an inspired $PO_2 < 30$ Torr. But these investigators found no change in reactivity to KCl and therefore concluded that it was unlikely that the supply of ATP available for contraction was limiting. A decrease in PO_2 results in membrane depolarization and an increased voltage-dependent permeability to Ca^{2+} (6). It is possible that, because the membrane is already partly depolarized by hypoxia, the amount of further depolarization due to other stimuli is limited, and hence the magnitude of the response to stimuli other than hypoxia is reduced. Another hypothesis for the decreased reactivity to NE is that hypoxia may result in the release of mediators that bind nonspecifically to and result in "heterologous" desensitization of the adrenergic receptors (10). Or such mediators might cause downregulation of the adrenergic receptors. Last, endothelium-derived factors such as endothelium-derived relaxing factor (NO) or endothelium-derived hyperpolarizing factor, reactive oxygen species, or NO from smooth muscle might be released in greater quantity after chronic hypoxia or a return to normoxia, thus compromising force development in response to high K^+or contractile agonists. The mechanisms for the decrease in responsiveness to NE during acute hypoxia and chronic hypoxia may or may not be the same. Important to note is that the literature is not in complete agreement about the effect of chronic hypoxia-induced pulmonary hypertension on NE reactivity, but this is likely due to differences in methodology. For example, McMurtry et al. (14) found an increase in response to NE in isolated perfused lungs from rats with chronic hypoxia-induced pulmonary hypertension. However, their data were not normalized and reflected the responses of the entire pulmonary circulation rather than those of isolated arterial muscle.

From the pilot studies with ANG II, it appeared that the peak response occurred at an ANG II dose of 10^{-7} M, and higher doses actually resulted in lower responses. Therefore, the range of doses was set at 10^{-10} to 10^{-6} M to ensure that a supramaximal dose was included for the purpose of demonstrating a maximum peak. However, the great variability in the magnitude for the responses of ANG II resulted in mean curves that do not have a clear maximum over the range of doses tested. When several higher doses were tested, the response was always submaximal. Similar dose ranges have been used by other investigators (13). It is worth noting that McMurtry et al. (13) did not use a supramaximal dose for ANG II, whereas Chand and Altura (2) did show a supramaximal response to ANG II at 10^{-7} M.

The ANG II dose-response curve of the hypertensive arteries is significantly shifted downward compared with the control curve, demonstrating a decrease in reactivity to ANG II in the arteries from rats exposed to chronic hypoxia. McMurtry et al. (13) also showed a decreased response to ANG II in both isolated perfused lungs and isolated main pulmonary arterial segments after exposure to chronic hypoxia. The possible explanations for the decrease in reactivity may be similar to those for NE, including heterologous (i.e., nonspecific) desensitization or receptor downregulation.

The 5-HT dose-response curve for the hypertensive PASM was not significantly different from that for the control PASM. 5-HT was the only agonist that did not lose its potency in eliciting a contraction as a result of chronic hypoxia. Downregulation of receptors with hypoxia may be receptor specific, or perhaps only certain intracellular coupling mechanisms are affected by chronic hypoxia.

Previously, Roepke et al. (26) reported that the predominant effect of Ado in the pulmonary circulation is vasodilation. Results of the present study show that hypertensive pulmonary arterial rings also relax in response to Ado. However, the relaxation induced by high doses of Ado was not as great in the hypertensive rings as in either endothelium-denuded or -intact control PASM rings. The decreased ability to relax in response to Ado may be contributing to the pulmonary hypertension.

Hypertensive rat pulmonary arteries show no difference in absolute maximum tension development (i.e., response to high K^+) from control arteries. This is surprising at first glance because pulmonary hypertension is associated with hypertrophy of the smooth muscle. McMurtry et al. (13) demonstrated that the pressor response to KCl is diminished in both isolated perfused lungs and isolated main pulmonary arterial segments from rats with chronic hypoxia-induced pulmonary hypertension. Because morphometric measurements in this study showed that there is an increase in CSA of the medial layer, it is best to normalize the KCl responses to the CSA. The finding that the CSA is doubled in the hypertensive vessels is supported by the fact that other investigators (7, 24) have shown a similar doubling of CSA. Results of this study show that the mean P_o normalized in this manner (i.e., P_o/CSA) is decreased in the hypertensive PASM. A decreased P_o/media CSA may be explained by an increase in the proportion of noncontractile to contractile smooth muscle (i.e., a phenotypic change) and/or a disproportionate increase in connective tissue in the hypertensive arteries.

Results of this study show the presence of four protein bands on SDS gels that closely correspond in molecular mass to those of myosin. The two densest bands (204 and 200 kDa) are the two forms of MHC (MHC_{200} and MHC_{204}, respectively) consistently found in a variety of mammalian smooth muscles (17, 18, 27, 29). Although these two MHCs are present in most smooth muscle tissues, the ratio of the amount of MHC_{204} to MHC_{200} varies depending on the specific smooth muscle tissue type from which myosin is isolated (16,29) and the animal's age (4, 15). Two other bands appear on the gels at the 190- and ~196- to 198-kDa positions. Degradation experiments have ruled out the possibility that the 190-kDa protein is a degradation product of MHC_{204} and MHC_{200}. Eddinger and Murphy (4) reported an MHC of 190 kDa that they isolated "most readily" from uterine tissue of the guinea pig and mouse, and it has also been isolated from aortic tissue from both of these species. These investigators claim that it has not been isolated from the rat, rabbit, or pig, although the rat pulmonary artery was not investigated. Another protein band that has a mobility intermediate between MHC_{200} and MHC_{190}, at ~196–198 kDa, has been reported (9, 27, 28). The 190- and 196- to 198-kDa MHCs have been described as nonmuscle MHCs and have been labeled nonmuscle myosin. Although in this study no change in the MHC_{200}/MHC_{204} ratio was found in the hypoxia-induced pulmonary hypertensive pulmonary arterial smooth muscle, an increase in the ratio of $MHC_{190+196}$ to $MHC_{200+204}$ in the hypertensive PASM did occur. Two clearly defined smooth muscle phenotypes have been reported: the contractile and the synthetic phenotypes (1). There is a relatively greater amount of nonmuscle myosin (i.e., MHC_{190} and MHC_{196}) in tissues from young animals, and the ratio of nonmuscle to muscle (MHC_{204} and MHC_{200}, respectively) myosin decreases with age (4, 15). An abundance of nonmuscle myosin may represent a less differentiated state of smooth muscle cells in the case of development or that more of the muscle is in the synthetic phenotype. On the other hand, expression of nonmuscle myosin could just as

easily be due to an increase in the number of nonmuscle cells, a possibility that cannot be contradicted without definitive staining in situ. However, a definitive immunohistochemical stain distinguishing synthetic smooth muscle from nonmuscle cells such as fibroblasts has not yet been identified.

In conclusion, the main findings of this investigation may be summarized as decreased reactivity, decreased tension production per unit of CSA of muscle despite hyperplasia, and an increase in the proportion of nonmuscle myosin to muscle myosin isoforms in the pulmonary arterial wall with pulmonary hypertension. The cause-and-effect relationship between these parameters remains to be established.

NOTES

- We thank Marlene Brown for expert typing of this paper and Peggy Harger-Allen and Sally Debono for excellent technical assistance in production of the micrographs utilized in this study.
- The work presented in this manuscript was funded by National Heart, Lung, and Blood Institute Grant HL-40894.
- J. E. Roepke was the recipient of an American Heart Association-Indiana Affiliate Fellowship. N. H. Oberlies was the recipient of an Indiana University School of Medicine Undergraduate Biomedical Research Program Scholarship.

REFERENCES

1. Chamley-Campbell, J., G. R. Campbell, and R. Ross. The smooth muscle cell in culture. *Physiol. Rev*. 59: 1–61, 1979.

2. Chand, N., and B. M. Altura. Reactivity and contractility of rat main pulmonary artery to vasoactive agents. *J. Appl. Physiol*. 49: 1016–1021, 1980.

3. Crouch, E. C., W. C. Parks, J. L. Rosenbaum, D. Chang, L. Whitehouse, L. Wu, K. R. Stenmark, E. C. Orton, and R. P. Mecham. Regulation of collagen production by medial smooth muscle cells in hypoxic pulmonary hypertension. *Am. Rev. Respir. Dis*. 140: 1045–1051, 1989.

4. Eddinger, T. J., and R. A. Murphy. Developmental changes in actin and myosin heavy chain isoform expression in smooth muscle. *Arch. Biochem. Biophys*. 284: 232–237, 1991.

5. Eddinger, T. J., and J. A. Wolf. Expression of four myosin heavy chain isoforms with development in mouse uterus. *Cell Motil. Cytoskeleton* 25: 358–368, 1993.

6. Fishman, A. P. The enigma of hypoxic pulmonary vasoconstriction. In: *The Pulmonary Circulation: Normal and Abnormal,* edited by A. P. Fishman. Philadelphia: University of Pennsylvania Press, 1990, p. 109–129.

7. Griffith, S. L., R. A. Rhoades, and C. S. Packer. Pulmonary arterial smooth muscle contractility in hypoxia-induced pulmonary hypertension. *J. Appl. Physiol*. 77: 406–414, 1994.

8. Harabin, A. L., M. D. Peake, and J. T. Sylvester. Effect of severe hypoxia on the pulmonary vascular response to vasoconstrictor agents. *J. Appl. Physiol*. 50: 561–565, 1981.

9. Kawamoto, S., and R. S. Adelstein. Characterization of myosin heavy chains in cultured aorta smooth muscle cells. *J. Biol. Chem*. 262: 7282–7288, 1987.

10. Lefkowitz, R. J., and M. G. Caron. Adrenergic receptors: molecular mechanisms of clinically relevant regulation. *Clin. Res*. 38: 395–406, 1985.

11. Lloyd, T. C., Jr. Influences of PO2 and pH on resting and active tensions of pulmonary arterial strips. *J. Appl. Physiol*. 22: 1101–1109, 1967.

12. Lowen, M. A., M. J. Bergman, M. V. Cutaia, and R. J. Porcelli. Age-dependent effects of chronic hypoxia on pulmonary vascular reactivity. *J. Appl. Physiol*. 63: 1122–1129, 1987.

13. McMurtry, I. F., K. G. Morris, and M. D. Petrun. Blunted hypoxic vasoconstriction in lungs from short-term high-altitude rats. *Am. J. Physiol*. 238 (*Heart Circ. Physiol*. 7): H849–H857, 1980.

14. McMurtry, I. F., M. D. Petrun, and J. T. Reeves. Lungs from chronically hypoxic rats have decreased pressor response to acute hypoxia. *Am. J. Physiol*. 235 (*Heart Circ. Physiol*. 4): H104–H109, 1978.

15. Mohammed, M. A., and M. P. Sparrow. Changes in myosin heavy chain stoichiometry in pig tracheal smooth muscle during development. *FEBS Lett*. 228: 109–112, 1988.

16. Murikami, N., E. Trenkner, and M. Elzinga. Changes in expression of nonmuscle myosin heavy chain isoforms during muscle and nonmuscle tissue development. *Dev. Biol*. 157: 19–27. 1991.

17. Packer, C. S. Changes in arterial smooth muscle contractility, contractile proteins, and arterial wall structure in spontaneous hypertension. *Proc. Soc. Exp. Biol. Med*. 207: 148–174, 1994.

18. Packer, C. S. Arterial muscle myosin heavy chains and light chains in spontaneous hypertension. *Comp. Biochem. Physiol. B Biochem. Mol. Biol*. 117: 19–28, 1997.

19. Packer, C. S., R. E. Bennie, D. R. Powell, and R. A. Rhoades. Neonatal hypoxia: long term effects on pulmonary arterial muscle. *Respir. Physiol*. 96: 213–229, 1994.

20. Packer, C. S., and R. A. Rhoades. Impaired pulmonary vascular smooth muscle function in lung injury. In: *Lung Vascular Injury,* edited by A. Johnson and T. J. Ferro. New York: Dekker, 1992, vol. 60, p. 227–262. (Lung Biol. Health Dis. Ser.)

21. Parks, W. C., R. P. Mecham, E. C. Crouch, E. C. Orton, and K. R. Stenmark. Response of lobar vessels to hypoxic pulmonary hypertension. *Am. Rev. Respir. Dis*. 140: 1455–1457, 1989.

22. Porcelli, R. J., and M. J. Bergman. Effects of chronic hypoxia on pulmonary vascular responses to biogenicamines. *J. Appl. Physiol*. 55: 534–540, 1983.

23. Porzio, M. A., and A. M. Pearson. Improved resolution of myofibrillar proteins with sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *Biochim. Biophys. Acta* 490: 27–34, 1977.

24. Reid, L. Structure and function in pulmonary hypertension: new perspectives. *Chest* 89: 279–288, 1986.

25. Rhoades, R. A., C. S. Packer, D.A. Roepke, N. Jin, and R. A. Meiss. Reactive oxygen species alter contractile properties of pulmonary arterial smooth muscle. *Can. J. Physiol. Pharmacol*. 68: 1581–1589, 1990.

26. Roepke, J. E., C. E. Patterson, C. S. Packer, and R. A. Rhoades. Response of perfused lung and isolated pulmonary artery to adenosine. *Exp. Lung Res*. 17: 25–37, 1991.

27. Rovner, A. S., R. A. Murphy, and G. K. Owen. Expression of smooth muscle and nonmuscle myosin heavy chains in cultured vascular smooth muscle cells. *J. Biol. Chem*. 261: 14740–14745, 1986.

28. Sartore, S., N. DeMarzo, A. C. Borrione, A. M. C. Zanellato, L. Saggin, L. Fabbri, and S. Schiaffino. Myosin heavy chain isoforms in human smooth muscle. *Eur. J. Biochem*. 179: 79–85, 1989.

29. Sparrow, M. P., A. Arner, P. Hellstrand, I. Morono, M. A. Mohammed, and J. C. Ruegg. Isoforms of myosin. In: *Regulation and Contraction of Smooth Muscle,* edited by M. J. Siegman, A. P. Somlyo, and N. L. Stephens. New York: Liss, 1987, p. 67–79.

30. Twarog, B. M., H. Takuno, S. Petrou, I. Wahlquist, R. Marcus, and G. L. Campbell. Pathogenesis of pulmonary hypertension in the rat model. *Chest* 93: 100s-101s, 1988.