Pulmonary artery smooth muscle activation attenuates arterial dysfunction during acute pulmonary hypertension

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Short title
PA smooth muscle role in acute hypertensive states

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Abstract
Acute pulmonary hypertension (PH) may arise with or without an increase in vascular smooth muscle (VSM) tone. Our objective was to determine how VSM activation affects both the conduit (CF) and wall buffering (BF) functions of the pulmonary artery (PA) during acute PH states. PA instantaneous flow, pressure and diameter of six sheep were recorded during normal pressure (CTL) and different states of acute PH, 1) passively induced by PA mechanical occlusion (PPH); 2) actively induced by intravenous administration of phenylephrine (APH); and 3) a combination of both (APPH). To evaluate the direct effect of VSM activation, isobaric (PPH versus APH) and isometric (CTL versus APPH) analyses were performed. We calculated the local BF from the elastic ($\eta_{\text{PD}}$) and viscous ($\eta_{\text{PD}}$) indexes as $\eta_{\text{PD}}/E_{\text{PD}}$, and the characteristic impedance ($Z_c$) from pressure and flow, to evaluate CF as $1/Z_c$. We also calculated the absolute and normalized cross-sectional pulsatility ($P_{\text{CS}}$ and $N_{\text{P}_{\text{CS}}}$, respectively), the dynamic compliance ($C_{\text{DYN}}$), the cross-sectional distensibility ($D_{\text{CS}}$), and the pressure-strain elastic modulus ($E_{p}$). The isobaric analysis showed increase of CF, BF and $\eta_{\text{PD}}$ ($P<0.01$) and decrease of $E_{\text{PD}}$ ($P<0.05$) during APH respect to PPH (concomitant with isobaric VSM activation-induced vasoconstriction, $P<0.01$). The isometric analysis showed increase of $E_{\text{PD}}$ and $\eta_{\text{PD}}$ ($P<0.01$), non significant difference in BF (even in the presence of a significant mean PA pressure rise, from 14±6 to 25±8 mmHg, $P<0.01$), and decrease in CF ($P<0.01$) during APPH respect to CTL. Mechanical occlusions (PPH and APPH) reduced BF ($P<0.01$), and increased $E_{\text{PD}}$ ($P<0.05$) with regard to their previous steady states (CTL and APH). Non significant differences were found in $E_{\text{PD}}$ between PPH and APPH. VSM activation (APH and APPH) increased $\eta_{\text{PD}}$ ($P<0.01$) respect to their previous passive states (CTL and PPH), but no significant differences were found within similar levels of VSM activation. In conclusion, VSM plays a relevant role in main pulmonary artery function during acute pulmonary hypertension, since isobaric vasoconstriction induced by VSM activation improves both BF and CF, mainly due to the increase in $\eta_{\text{PD}}$ concomitant with the arterial compliance. $C_{\text{DYN}}$ and $D_{\text{CS}}$ were the more pertinent clinical indexes of arterial elasticity. Additionally, the $\eta_{\text{PD}}$-mediated preservation of the BF could be evaluated by the geometric related indexes ($P_{\text{CS}}$ and $N_{\text{P}_{\text{CS}}}$), which appear to be qualitative markers of arterial wall viscosity status.

Key Words
Buffering function, arterial wall viscoelasticity, characteristic impedance.

Glossary
- $\eta_{\text{PD}}$: viscous index
- APH: active pulmonary hypertension
- APPH: active plus passive pulmonary hypertension
- BF: buffer function
- $C_{\text{DYN}}$: dynamic compliance
- CF: conduit function
- CTL: control
- $D_{\text{CS}}$: cross-sectional distensibility
- $E_{\text{INC}}$: incremental elastic modulus
- $E_{p}$: pressure-strain elastic modulus
- $E_{\text{PD}}$: elastic index
- $N_{\text{P}_{\text{CS}}}$: normalized cross-sectional pulsatility
- PA: pulmonary artery
- $P_{\text{CS}}$: cross-sectional pulsatility
- PH: pulmonary hypertension
PPH: passive pulmonary hypertension
VSM: vascular smooth muscle
$Z_C$: characteristic impedance
Introduction

At present, there is worldwide interest in clinically assessing the local mechanical properties of the pulmonary artery (PA) during pulmonary hypertensive states (7, 10, 34). Although measurements of pressure, mean flow and/or pulmonary vascular peripheral resistance remain as the “gold standard” for hemodynamic evaluation during pulmonary hypertension (PH), they do not suffice to evaluate the local properties of the PA wall (10). Recently, important progresses have been made with sophisticated techniques allowing PA diameter (or cross sectional area) to be continuously tracked throughout the systolic-diastolic cycle, sometimes with concomitant accurate continuous blood pressure measurements from nearby sites (7, 10, 34, 38). Thus, the diameter, flow and/or pressure temporal signals allow the calculation of several mechanical parameters, but some problems still exist when using them. Firstly, with those indexes it is not possible to separate dynamically the elastic from the muscular component of the PA wall. Secondly, there is no information about the intrinsic effect of those components on the main functions of large arteries, namely, to conduct blood (conduit function, CF) and to buffer pulsatility (buffer function, BF) (28). And third, whether the mechanical indexes can distinguish between changes in the arterial function due to vascular smooth muscle (VSM) activation and to changes in the arterial pressure during PH, remains to be established.

Regarding the etiology, physiopathology, and pulmonary hemodynamic state, PH has been classified into numerous subclasses (36). When considering the pulmonary VSM tone, two states can be grossly differentiated: “passive” an “active” PH (33). The former includes those instances in which there is an acute elevation of the arterial pressure with a passive distension of the arterial wall and without important changes in VSM activity (i.e. PH induced by diastolic left ventricular failure, pulmonary vascular obstruction) (33). On the contrary, active PH is applicable to situations that the arterial wall, while supporting a passive force of distension, executes active contraction (i.e. PH due to an increase in sympathetic autonomic tone, hypoxic vasoconstriction, and/or local neurohumoral mediators) (33).

Pulmonary arteries, in contrast with systemic arteries, have a much thinner smooth muscle layer under normal conditions, consistent with a low-pressure system, and, as an approximation, the pulmonary vascular resistance is divided equally between arteries, capillaries and veins (3). This explains why the effects and physiological role of VSM tone on pulmonary CF and BF during PH still remain controversial (33). The controversy is mainly due to the difficulty in separating the direct effects that a vasoactive drug exert on the arterial wall from the indirect effects caused by an increased blood pressure and/or arterial diameter. Previous studies performed on other vascular beds (1, 2, 4, 5) and in the PA (8, 9, 18) have evidenced that VSM activation reduces isobarically the elastic index ($E_{PD}$) during acute hypertension. However, to our knowledge, the effect of VSM activation on the CF and the BF of the PA in vivo, and their relationship with the most commonly used clinical indexes have not been reported.

Our aim was to characterize elastic and muscular behavior of the PA wall by using a viscoelastic model, and to compare in a dynamically isobaric and isometric approach the effects of VSM activation on the CF and the BF in a sheep model of acute PH. Additionally, we analyzed the effects of passive and active PH over several widely used arterial function parameters. This would allow to evaluate the ability of commonly used indexes to detect pressure-dependence and VSM activation-dependence in the PA mechanical behavior during acute PH.
**Materials and methods**

All procedures agreed with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Research Council (National Academy Press, Washington, D.C. 1996).

**Surgical preparation**

Six Merino sheep weighing 26 to 30 kg were operated. Anesthesia was induced with intravenous sodium pentobarbital (35 mg·kg\(^{-1}\)) and maintained with additional pentobarbital as required. Animals were intubated and ventilated with a positive pressure respirator (Dragger SIMV Polyred 201, Spain). Respiratory rate and tidal volume were adjusted to maintain arterial pCO\(_2\) at 35-45 mmHg, pH at 7.35-7.4, and pO\(_2\) above 80 mmHg. The right saphenous vein was catheterized to administrate saline solution, anesthesia and phenylephrine. A pressure microtransducer (Millar Mikro-tip, SPC 370 7F) was inserted through the femoral artery and placed at the level of the abdominal aorta to monitor systemic pressure. The PA and its main branches were exposed by thoracotomy at the left fifth intercostal space.

A nonconstricting ultrasonic perivascular flow probe (Transonic Systems, Ithaca, NY, USA) positioned around the main pulmonary artery 2 cm downstream of the pulmonary valve allowed instantaneous measurement of pulmonary flow. A solid state pressure microtransducer (model P7, Konigsberg Instruments, Inc., Pasadena, CA, USA), previously calibrated using a mercury manometer, was inserted in the PA through a little incision, just distal to the flow probe. In order to measure the PA external diameter, two miniature piezoelectric crystal transducers (5 MHz, 2 mm in diameter) were sutured on opposite sites in the adventitia (Figure 1). After instrumentation, the chest was closed, but no attempt was made to restore a negative pleural pressure.

The external pulmonary diameter signal was calibrated in mm using the 1 mm step calibration facility of the sonomicrometer (model 120, Triton Technology Inc, San Diego, CA, USA). The transit time of the ultrasonic signal (velocity=1580 m·s\(^{-1}\)) was converted into distance (diameter) by means of the sonomicrometer. Both the sonomicrometer and the flowmeter were synchronized. Therefore, instantaneous flow, pressure and diameter signals were recorded simultaneously and at the same site of the PA. In order to induce acute increases in pulmonary arterial pressure, a pneumatic occluder was placed around the left branch of the PA, 5-6 cm distal from the ultrasonic crystals, ensuring that no artifacts appeared in diameter measurements during pulmonary artery occlusions.

**Experimental protocol**

After surgical instrumentation, instantaneous flow, pressure and diameter signals of the PA were recorded (sampling rate: 200 Hz) during 4 hemodynamic states, with the ventilation paused at end-expiration:

1) Control state of normal pressure (CTL): steady state without drug administration and/or mechanical occlusion.

2) Active pulmonary hypertension (APH): achieved by activation of the VSM by intravenous infusion of phenylephrine (5 µg·kg\(^{-1}\)·min\(^{-1}\)).

3) Passive pulmonary hypertension (PPH): achieved by occlusion of the left PA (5 seconds maximum) until the pressure of the main PA reached similar values to those encountered during administration of phenylephrine.

4) Active plus passive pulmonary hypertension, APPH: achieved by occlusion of the left PA (5 seconds maximum) during APH state, until the diastolic diameter of the main PA reached similar values than CTL.

In order to re-establish control values of signals, 10 minutes were allowed to elapse between the hypertensive maneuvers. The similarity between the arterial pressure levels during active and passive situations enabled us to perform an isobaric analysis (APH vs. PPH,
Figure 2). Likewise, the similarity between arterial diameter levels during the CTL and APPH enabled us to complete the study with an isometric analysis (CTL vs. APPH, Figure 2). At the end of the experiment, the sheep was killed by an overdose of sodium pentobarbital. The PA was removed and the correct location of the diameter and pressure sensors was confirmed. In order to determine the wall thickness of the PA, an arterial segment 4-5 cm long, previously measured in vivo, was excised and weighed.

**Calculus**

Approximately 15-20 consecutive beats were analyzed in each hemodynamic steady state (CTL and APH). During transient states (PPH and APPH) only 3-4 beats within the occlusion maneuver were considered.

**Incremental elastic modulus and wall stiffness indexes**

PA wall thickness, strain ($\varepsilon$) and stress ($\sigma$) were calculated as previously reported (1, 4) using the following equations, assuming a thick-walled cylinder with constant length:

$$\varepsilon = \frac{R}{R_0}$$

$$\sigma = \frac{2P(r_e^{-2} - r_i^{-2})}{r_e^{-2} - r_i^{-2}} \cdot \frac{1}{R^{-2}}$$

where $R$ is the midwall radius calculated as $R = (r_e + r_i) / 2$, $R_0$ is the non stressed midwall radius measured during autopsy approximately at 0 mmHg of PA pressure ($P$), $r_e$ is the measured PA external radius, and $r_i$ is internal radius calculated as:

$$r_i = \sqrt{r_e^2 - \frac{V}{\pi \cdot L}}$$

where $V$ was calculated using the weight of a given wall segment of *in vivo* length $L$, assuming a density of 1.06 g·cm$^{-3}$. Because $V$ does not change in vivo, $r_i$ and wall thickness of the vessel can be calculated continuously (1, 4).

The incremental elastic modulus ($E_{INC}$), a true evaluator of the elastic status of the vessel wall from the stress-strain relationship, was assessed assuming the linear elastic theory and the PA wall as an isotropic homogeneous elastic material, according to the following equations:

$$E_{INC} = 0.75 \frac{d \sigma}{d \varepsilon}$$

where $d\sigma/d\varepsilon$ represents the first derivative of stress respect to strain. $E_{INC}$ conceives the vessel as a hollow structure, and provides information about the wall artery material regardless from its geometry and/or size.

**Parameters derived from single systolic and diastolic values**

The pressure-strain elastic modulus ($E_p$) was calculated as (28, 32):

$$E_p = D_D \frac{(P_S - P_D)}{(D_S - D_D)}$$

where $P_S$, $P_D$, $D_S$ and $D_D$ are the systolic and diastolic values of the pulmonary pressure and diameter, respectively. The pulmonary pulse pressure was calculated as $P_S - P_D$.

The dynamic compliance ($C_{DYN}$), which involves the inverse of the $E_p$ (and therefore $\approx 1/E_{INC}$), was calculated as (40):

$$C_{DYN} = \frac{(D_S - D_D)}{D_D(P_S - P_D)} \times 10^4$$

The cross-sectional pulsatility ($P_{CS}$) and the normalized cross-sectional pulsatility ($NP_{CS}$) were calculated as (7, 10, 34):

$$P_{CS} = \text{systolic area} - \text{diastolic area}$$
Cross-sectional distensibility ($D_{CS}$) was calculated as (7):

$$D_{CS} = \frac{NP_{CS}}{(P_S - P_D)}$$

**Viscoelastic model**

The viscoelastic properties and the buffering capacity of the arterial wall were studied using a Kelvin-Voigt viscoelastic model (spring-dashpot). According to this, the total pressure developed in the wall ($P_{total}$) to resist stretching can be divided into viscous ($P_{viscous}$) and elastic ($P_{elastic}$) components (1, 9):

$$P_{total} = P_{elastic} + P_{viscous}$$

As viscous pressure is proportional to the first derivative of the arterial diameter with respect to time ($dD/dt$), $P_{elastic}$ can be expressed as:

$$P_{elastic} = P_{total} - \eta_{PD} \frac{dD}{dt}$$

where $\eta_{PD}$ is the viscous index of the arterial wall. In order to separate the purely elastic wall properties, the viscous term must be subtracted from $P_{total}$, finding the optimal value through the criterion of disappearance of the hysteresis loop. Increasing values of $\eta_{PD}$ were given by visually inspecting the reduction of the hysteresis loop area. When the area reached a minimum (considered as the value that preserved the clockwise course of the loop), the elastic index ($E_{PD}$) was calculated as the slope of the elastic pressure-diameter curve at the mean diastolic pressure (1, 9):

$$E_{PD} = \frac{dP_{elastic}}{dD}_{mean \ diastolic \ pressure}$$

A decrease in $E_{PD}$ indicates a decrease in elasticity and hence a reduction of the stiffness of the arterial wall or an increase in compliance.

**Conduit function (CF)**

The CF of the PA was evaluated by means of the local hemodynamic impedance. It was quantified in terms of the characteristic impedance ($Z_C$). $Z_C$ is defined as the impedance in the absence of reflected waves and correlates directly with the elastic properties and inversely with the cross-sectional area of the vascular bed according to the Water-Hammer formula (22). An increased $Z_C$ will determine an augmented impedance against blood flow (12) resulting in decreased capacity to conduct blood from the heart to the vessels while maintaining an elevated intraluminal pressure, necessary to overcome the vascular peripheral resistance. Therefore, by inverse reasoning, the CF was computed as $1/Z_C$. Pulmonary vascular impedance was calculated from the Fourier series expressions for pressure and flow signals (28). Between three and six end-expiratory heartbeats were analyzed for each data-collection interval. Pressure and flow harmonics with amplitudes of 1% of pressure and flow pulse amplitude were excluded from pulmonary vascular impedance calculations. The pulmonary vascular impedance modulus was computed as the ratio between pressure and flow moduli, and its phase was computed as the difference between pressure and flow phases. $Z_C$ was calculated as the average of impedance moduli between 2 and 15 Hz.

**Buffering function (BF)**

Recently, our group proposed the characterization of wall BF by means of the arterial wall time constant, obtained as the ratio $\eta_{PP}/E_{PD}$ when a Kelvin-Voigt model represents the arterial wall (8). Accordingly, the P-D relationship was established using Eq. 1, Eq. 2 and the computed $E_{PD}$ and $\eta_{PD}$
\[ P(t) = P_0 \quad t > 0 \quad \Rightarrow \quad D(t) = \frac{P_0}{\eta_{PD}/E_{PD}} \left( 1 - e^{-\frac{t}{\eta_{PD}/E_{PD}}} \right) \quad t > 0 \]

where the \( \eta_{PD}/E_{PD} \) ratio would characterize the exponential temporal response of diameter due to a pressure change. This ratio, the time constant of the Kelvin-Voigt model or “time retardation” (42), describes the temporal response of arterial diameter following acute variations of pressure (creep response or relative damping effect). An elevated value of BF is related with a slow response, suggesting an augmented buffering effect with an increased attenuation of pressure oscillations.

**Statistical analysis**

Both measured and calculated values were expressed as mean ± standard deviation. Significant differences between values were assessed using ANOVA for repeated measures, followed by a Bonferroni test for multiple comparisons. Statistical significance was set at \( P < 0.05 \). Uncertainty was calculated as:

\[ u = \sqrt{B^2 + (t_{(v,0.95)} \times P)^2} \]

where B is the measurement bias estimate derived from error propagation of index formulae, P is the measurement precision estimate derived from standard error, and \( t_{(v,0.95)} \) is the t value calculated from a Student t distribution with v degrees of freedom and 95% confidence (40).
Results

Table 1 shows the hemodynamic parameters for the four experimental states. Heart rate showed no significant differences between them. During both PPH and APH, diastolic, systolic and mean pressures were alike enabling an isobaric comparison. During CTL and APPH, diastolic and mean diameters were similar, enabling an isometric comparison. The significantly decrease in mean and diastolic diameter during APH respect to CTL ($P<0.05$) and PPH ($P<0.01$) evidenced the vasoconstriction due the administration of phenylephrine.

Figure 2 shows the individual PA pressure-diameter loops corresponding to each experimental state in the six sheep. Notice that phenylephrine administration (APH) increased pressure with concomitant reduction in diameter in all animals, shifting the loops upwards and to the left. Furthermore, APH pressure-diameter loops appear virtually isobaric respect to passive mechanic occlusion (PPH). Contrarily, loops from control and mechanical occlusion during VSM activation (APPH) are virtually isometric.

Figure 3 shows the calculated viscoelastic parameters and the functional ones, CF and BF. The isobaric analysis of CF, BF and $\eta_{PD}$ showed that the APH values were higher ($P<0.01$) and $E_{PD}$ lower ($P<0.05$) than those during PPH, concomitant with arterial vasoconstriction (from $23.9\pm2.2$ to $22.3\pm2.4$ mm in PA mean diameter, $P<0.01$) induced by VSM activation.

When comparing viscoelastic parameters ($E_{PD}$, $\eta_{PD}$) isometrically, APPH showed higher values than CTL ($P<0.01$). No significant differences were found in BF, between the APPH and CTL (isometric comparison) even at the highest pressure level ($14\pm6$ versus $25\pm8$ mmHg in PA mean pressure, $P<0.01$) while CF decreased ($P<0.01$) with respect to CTL.

During both states induced by acute mechanical occlusions (PPH and APPH), BF was smaller ($P<0.01$), and $E_{PD}$ higher ($P<0.05$), than their previous steady states (CTL and APH). No significant differences were found in $E_{PD}$ between PPH and APPH. During both active states (APH and APPH) $\eta_{PD}$ increased ($P<0.01$) when compared with their previous passive states (CTL and PPH), but no significant differences were found within similar level of VSM activation.

Table 2 shows the $E_{INC}$ (considered as a true evaluator of the elastic status of the vessel wall) and a variety of clinical indexes derived from single systolic and diastolic measurements of pressure and/or diameter (or cross sectional area) widely used for the evaluation of the biomechanics of pulmonary hypertension. Notice that only the indexes that relate to both pressure and diameter values ($C_{DYN}$, $D_{CS}$ and $E_{P}$) show parallel qualitative changes as $E_{INC}$, in all the hemodynamic states. On the other hand, cross sectional pulsatility and normalized cross sectional pulsatility show similar trend as the viscous index. Finally, pulse pressure follows $E_{INC}$ only during passive conditions.

In order to evaluate the usefulness of the parameters which follow $E_{INC}$, a standard engineering uncertainty analysis was performed for the $E_P$, $C_{DYN}$ and $D_{CS}$ (Table 3). Mechanical pressure increases provoke augmentation in $E_P$ uncertainty and diminution in both $C_{DYN}$ and $D_{CS}$ uncertainties. $E_P$ uncertainty was always higher (ranged between 62% to 91%) than that corresponding to $D_{CS}$ (ranged between 49% and 54%) and $C_{DYN}$ (ranged between 48% to 54%).
Discussion

The purpose of this work was to characterize, to compare and to analyze, in isobaric and isometric ways during passive and active acute PH states, the effects of VSM activation on (a) the CF and BF of the PA, and (b) a variety of widely used parameters of arterial function. The following discussion focuses on five central conclusions of this study.

(1) VSM contraction during isobaric acute PH does not necessarily yield the “expected” increase in pulmonary characteristic impedance (due to a cross-sectional area reduction) with concomitant reduction of the CF and BF. Inversely, VSM activation determines an isobaric improve in CF and BF.

(2) VSM activation during isometric maneuvers increases the arterial elastic index, maintaining the BF near the control values through a genuine increase in wall viscosity.

(3) The viscous index ($\eta_{PD}$) possibly would be a pressure-independent sign of the degree of VSM activation.

(4) Elastic indexes that involve pressure and diameter signals ($C_{DYN}$, $D_{CS}$ and $E_{P}$) allow a qualitative and comparative analysis during different hypertensive states, and could be used to detect pressure- and/or activation-dependent changes, exclusively, in arterial elasticity.

(5) $C_{DYN}$ and $D_{CS}$ appear to be the most reliable indexes to extrapolate $E_{INC}$ in order to evaluate PA hypertensive states from single systolic-diastolic values.

Arterial conduit function

The CF of the main arteries allows blood transfer from the heart to the peripheral vessels while maintaining an elevated intraluminal pressure, necessary to overcome the vascular peripheral resistance. To maintain an adequately high level of mean pressure and to minimize ventricular work, low arterial impedance must be presented to the pulsatile blood flow ejected by the heart (31). This is possible because the main PA has a large cross-sectional area (lumen) with distensible wall, adaptable to ventricular ejection.

Controversial results have been obtained when determining $Z_{C}$ during acute PH. Several authors have found an increase in $Z_{C}$ during acute PH induced by an abrupt proximal obstruction of the pulmonary arterial tree. This effect mainly results from the reflected waves that come back during systole (before closure of the pulmonary valve) and thus directly oppose blood ejection during ventricular contraction (11, 15, 16, 23, 26, 39). Oppositely, previous studies regarding acute PH induced by lung injuries, such as the injection of small (150 to 200 $\mu m$) glass spheres (11, 16, 23, 27), injury with oleic acid (11, 29) and during autologous blood clot embolism (23), reported an increase in peripheral resistance, with either stable or decreased $Z_{C}$.

Acute changes in arterial geometrical and/or elastic properties (determinants of $Z_{C}$) are due to the mechanical effect of arterial pressure variations and/or functional modifications of vascular tone (8). For this reason, in this work we performed an isobaric and isometric analysis. Our results showed that the isobarically calculated $Z_{C}$ was lower during APH, than during PPH, in spite of the diminished diameter resulting from isobaric vasoconstriction during APH. This situation was counterbalanced by a significant reduction in arterial wall elastic index (i.e. more compliant) during APH with respect to PPH (Figure 3 and Table 2). Therefore, the interrelation between geometric and intrinsic wall variables suggests that VSM activation is responsible for the isobaric preservation of the CF, regardless from arterial vasoconstriction. The essential role of the arterial wall can be clearly established when comparing PPH and CTL hemodynamic states. Notice that, in spite of an increased mean diameter due to pressure overdistension, the increasing wall elastic index during PPH causes a decrease in the CF (higher $Z_{C}$) with respect to the state of normal pressure (CTL).

Recently, Wauthy et al (39) reported in dogs and goats that the isobaric increase of $Z_{C}$ is more pronounced during mechanical occlusion of the PA than during hypoxia or pulmonary embolism, with concomitantly decreased PA compliance. Given that both hypoxia and
embolism induce vasoreactivity (17, 33) our results agree with those of Wauthy et al. We show that VSM activation of the PA reduces ventricular afterload under isobaric conditions by reducing $E_{INC}$ and $Z_C$. This, in turn, improve the energy transfer between the right ventricle and the pulmonary circulation (29).

**Arterial wall buffering function**

One of the most important functions of the elastic properties of the arterial wall is to store part of the mechanical energy generated by the heart during systole, and to restore it in diastole, optimizing the heart-vessel coupling and ensuring a continuous flow toward the tissues (28). Other part of this energy is dissipated by means of the viscous properties of the arterial wall (1). At date, it is accepted that the arterial wall BF is determined not only by arterial elastic properties, but also by the viscous properties of the wall (8, 9, 41). Hence, a proper mechanical characterization of the arterial BF must consider both wall elasticity and viscosity.

The viscous behavior is thought to represent the contribution of the VSM to the behavior of the arterial wall (1, 8, 9, 14, 41). Our results during pulmonary VSM activation agree with those of Patel et al (30), Ingram et al (19), and Cox (14), in canine pulmonary arteries. Although these in vitro studies evidenced an increase in the viscosity of the PA during VSM activation, the functionality of this has not yet been analyzed (14). Our results evidence that pulmonary VSM activation would determine the augmented energy dissipation due to wall viscosity, and they agree with those reported by Armentano et al (1) and Barra et al (5) in the aorta of dogs with acute hypertension. During acute PH, we found (a) a higher viscosity during "active" conditions (APH and APPH) with respect to "passive" conditions (CTL and PPH); and (b) similar values of $\eta_{PD}$ within pre and post mechanical occlusions (CTL vs. PPH, and APH vs. APPH). These findings support the hypothesis that there is a relationship between $\eta_{PD}$ and VSM activation, and that $\eta_{PD}$ is independent of acute changes in arterial pressure, and allow us to explain the pulmonary vasoconstriction by a direct effect of phenylephrine on VSM, minimizing the co-existence of a myogenic response. The myogenic response has been found in a wide variety of vessels with intermediate diameter from different vascular beds, and vessel wall tension has been identified as the stimulus for the myogenic response. However, larger and very small vessels possess a relatively weak myogenic response (35). There is little published information regarding the myogenic response in pulmonary arteries. Kulik et al (21) demonstrated in isolated feline pulmonary arteries that, in contrast with segments from small pulmonary arteries (<1000 $\mu$m), segments from vessels with an in situ diameter of >1000 $\mu$m did not contract with stretch.

Considering wall viscosity as a pressure-independent index of the degree of VSM activation, we would be able to determine whether the level of arterial CF and/or BF are or not due to changes in VSM activation.

In the present work the decrease in diameter (Table 1) evidenced the activation of VSM during APH. Diameter and $E_{INC}$ variations, together with the results of previous studies (1, 4) characterizing the aortic wall behavior, would lead us to hypothesize that the reduction of pulmonary diameter induced by VSM activation would reduce the recruitment of collagen fibers. Thus, wall stress would be transferred from collagen fibers to VSM. Contrarily, PPH causes a pressure elevation that distends the vessel, recruiting collagen fibers and augmenting arterial stiffness. It could be said that both elastin and collagen contribute to arterial stiffening in a passive way. Active wall response would be modulated by the degree of VSM activation during PH, independently of the effects caused by the level of pressure. In agreement with our results, Cox observed in canine pulmonary arterial rings that VSM activation shifts the stress-strain (or P-D) curve upwards and to the left (13).

To analyze BF, the relative damping effect, the $\eta_{PD}/E_{PD}$ time constant was calculated. Our results showed a diminished $\eta_{PD}/E_{PD}$ value during PPH with respect to CTL and APH.
This result was due to an increasing E_{PD} with stable \eta_{PD}, and consequently BF was reduced. However, \eta_{PD}/E_{PD} was higher during APH, at equal levels of pressure than those encountered in PPH (isobaric analysis). This would suggest that a change in viscosity during activation would be vital for maintaining the arterial cushioning. The BF was similar during CTL and APPH (isometric analysis) despite the higher levels of pressure and elasticity observed in APPH. Therefore, both the isobaric and the isometric analysis evidenced a beneficial effect of VSM activation consisting of an enhancement of the arterial BF. In this way, the vascular system is able to attenuate the acute changes produced by PH, maintaining or even augmenting the arterial buffering capacity.

Arterial wall mechanical index

In the clinical setting, the hemodynamic characterization of the pulmonary circuit and its response to vasoactive drugs is done by applying Poiseuille's law from pressure and cardiac output determinations (6). However, these measurements have several limitations: a) they only assess the status of the stationary component of the pulmonary vasculature, neglecting the essentials of a pulsatile circulation (7); b) they are weakly correlated with histological findings; c) they have a poor prognostic value; and d) they do not have ability to evaluate both the structural and functional states of specific segments of the pulmonary vasculature (10).

Recently, invasive techniques measuring instantaneous diameter signals (i.e., intravascular ultrasound) have been used to evaluate particular segments of the pulmonary vasculature. Some of these techniques even allow simultaneous determination of arterial pressure and/or diameter signals. Quantitative and qualitative changes in each of these parameters during passive or active states may depend on the geometric, intrinsic, and/or peripheral effects of VSM activation (2, 25).

According to our results, the incremental elastic modulus (characteristic parameter of the intrinsic elasticity of the material, independently of its size and geometry) followed the same tendency as the elasticity calculated from the P-D relationship (E_{PD}). The isobaric analysis demonstrated a reduction of these parameters during VSM activation, whereas isometrically, they increased. Moreover, their values were similar to those previously found by Ingram et al (19) in dogs (1.7×10^6 dyn·cm^{-2} in the main PA at 20 mmHg) and by Cox (13) on intra and extralobar canine pulmonary arterial ring segments (static elastic modulus of 1.0-1.5×10^6 dyn·cm^{-2}). Parameters derived from single systolic-diastolic values of arterial diameter and pressure, such as E_P, C_{DYN} and D_{CS}, showed a similar qualitative behavior.

The behavior of the PP was not similar to that encountered when using either intrinsic mechanical properties or indexes relating pressure and diameter signals. Besides, indexes that depend only on arterial diameter signals (P_{CS} and N_{P_{CS}}) did not follow the same patterns as E_{INC}. Moreover, neither of them differed significantly during passive states (CTL vs. PPH) and during active states (APH vs. APPH). So, absolute or normalized values of arterial pulse diameter could be used as indicators of the degree of VSM activation. Only during VSM activation arterial pulse diameter and wall viscosity increased with regard to the passive situation, independently from the pulse pressure level, suggesting that pulse diameter could be an indirect marker of arterial wall viscosity.

Uncertainty is a critical parameter for evaluation of any measurement technique. The uncertainty of a parameter stems from both the experimental equipment (bias) and data scatter (precision). In our case, precision uncertainties were always higher than bias uncertainties because of the data scatter inherent to the nature of the animal model. E_P presented a high uncertainty value explained by its inverse dependence on the change in diameter. As was stated by Weinberg et al, C_{DYN} (and D_{CS}) should be a more systematically reliable parameter to describe wall alterations induced by hypertension (40).
Clinical Implications

Although the results of the present study apply to acute PH situations and therefore should not be extrapolated to chronic hypertensive vascular disease, they provide some evidence about the role that the VSM of the PA could play in patients with different types of PH in order to maintain the functionality of the pulmonary circulation. Our results show that VSM activation in the PA determines substantial changes in function even when working under similar levels of mean and pulse pressure. When analyzing the results obtained under active and passive PH, it is evident that not all acute PH states represent the same conditions of work for the cardiovascular system as a whole, as well as for the right ventricle and PA in particular. VSM tone increase would augment wall viscosity, improving both the CF and BF. This isobaric improve in CF and BF of the PA results in important benefits for the cardiovascular system: a) it favors ventriculo-arterial coupling, by reducing the total RV hydraulic load; b) it reduces stress fatigue in the arterial wall of the great arteries, and c) it ensures continuous low-pressure flow in the microcirculation. Thus, under diverse states of PH, VSM activation would help minimize the deleterious effects of cardiac and vascular overload. Acute PH causes significant passive changes in the mechanical behavior of the main PA and an increased right ventricular (RV) afterload (9). Given that RV failure is the main cause of death in PH, a better understanding of the factors determining RV afterload is crucial. Clinical signs of RV failure vary greatly in presence of similar severity of PH as evaluated by pulmonary pressure measurements and resistance calculations. Pulmonary vascular resistance or pressure are insufficient to evaluate RV afterload (7). The P-D arterial characterization of the PA provides important diagnostic and therapeutic information, as it already does in the systemic circulation (37).

The elastic incremental modulus (E\text{INC}, a true evaluator of the elastic status of the vessel wall from the stress-strain relationship), and clinical indexes derived from single systolic-diastolic values of pressure and/or diameter showed that only indexes relating pressure and diameter (C\text{DYN}, D\text{CS}, and E\text{P}) followed qualitative changes as did E\text{INC}, in all hemodynamic states. Further studies are necessary to show the applicability of the C\text{DYN} and viscous index to chronic experimental or clinical pulmonary hypertension.

Study significance and limitations

The experimental model was designed to study the direct (pressure- and volume-independent) role of the PA vascular smooth muscle during acute PH. By phenylephrine infusion and/or transient mechanical occlusion of the left PA, we generated isometric and isobaric states with and without activation of the main PA smooth muscle. During phenylephrine infusion (APH and APPH) both large and small pulmonary vessels contracted. Thus, the pressure increase recorded in the main PA was due to both effects, while during APPH, PA pressure was further increased by transient mechanical obstruction of the left PA.

On the other hand, by using mechanical occlusion during PPH, we fulfilled the objective of generating PH without increasing VSM tone, but isobaric with respect to APH. Our aim was not to mimic PH secondary to elevation in left ventricular diastolic pressure (i.e. left ventricular acute diastolic failure) or secondary to left atrial hypertension (i.e. mitral valve disease), but to generate a state of passive PH. It has been shown that in the early moments of such states the pulmonary artery pressure increase would be “passive”, caused by the resistance to pulmonary venous blood flow and the resulting retrograde transmission of the hypertension initiated in the left chambers of the heart (33). The term “passive” refers to the fact that during these early instants no relevant increment of pulmonary smooth muscle tone occurs. On the other hand, it has been shown that when this situation evolves, VSM tone in the peripheral pulmonary vessels increases (33). Consequently, terming “passive hypertension” the early stages of this mechanically induced hypertension is a simplification
only used to exemplify a condition where passive arterial overdistention with little increase of VSM tone is to be expected.

In most forms of pulmonary hypertension a neurally or humorally mediated VSM tone increase exists (17, 24, 33). Depending on the etiology, VSM activation would occur earlier (e.g., hypoxemic pulmonary hypertension) or later (e.g., pulmonary hypertension associated with hepatic disease). During pulmonary hypertension caused by acute hypoxia, pulmonary artery pressure increases due to activation of VSM induced by angiotensin II and endothelin, among other factors (24). Contrarily, during massive or moderated pulmonary embolism, mechanical factors would be responsible for the early increase in pulmonary vascular resistance and hence of pulmonary hypertension (17). In these cases, the release of vasoconstricting compounds, reflex pulmonary artery vasoconstriction, and hypoxemia may further increase pulmonary vascular resistance and result in a more severe PH (17).

During certain stages of primary PH, alterations of the control mechanisms of VSM tone determining increased pulmonary vasoreactivity exist (33). In addition, the autonomous nervous system, through neural stimulation or catecholamine release, as well as elevated levels of circulating endothelin increase arterial pressure (33). In occasions, partial or total vascular occlusion provoked by pulmonary artery remodeling and/or thrombosis further contributes to pressure increment (33). Thus, VSM activation may be considered the common final stage of the diverse forms of PH. In other words, regardless from its nature (acute or chronic) and pathogeny (passive or active), PH will exhibit increased VSM activity early after its onset. Although VSM activation in the small peripheral vessels determines a greater increase in pulmonary pressure, our results suggest that VSM activation in the large pulmonary vessels may probably play a role. It may be speculated that VSM activation of the large pulmonary conduits is an adaptative mechanism of right cardiovascular function, shared by the diverse forms of PH. If isobaric VSM activation fails to occur, a lesser cardiovascular adaptation capacity to increased pulmonary artery pressure is to be expected.

Conclusions

In conclusion, VSM appears to be a relevant component of the wall in order to improve the function of the main pulmonary artery during acute pulmonary hypertension. In effect, vasoconstriction induced by VSM activation improves both buffering and conduit function of the PA, mainly due to the increase in wall viscosity concomitant with the arterial compliance. Our study indicates that $C_{DYN}$ as well as $D_{CS}$, the clinical indexes of arterial elasticity with lowest uncertainty, were the most adequate clinical indexes of arterial elasticity. Additionally, isometric VSM contraction preserves BF mediated by arterial wall viscosity increase despite of the strong increment in PA pressure. In all cases, geometric related indexes ($P_{CS}$ and $NP_{CS}$) appear to be qualitative markers of the arterial wall viscosity status, and therefore, evaluators of smooth muscle tone.
Acknowledgements

This work was performed within a cooperation agreement between Universidad de la República (Uruguay)-Universidad Favaloro (Argentina), and it was partially supported by Fondo Clemente Estable (DINACYT) Nº 8284, PEDECIBA, and the René Favaloro University Foundation. The authors gratefully acknowledge the technical assistance of Mr. Elbio Agote and Ms. Edith Moraes, and Dr. Alberto Crottogini for revising the manuscript.
References


Table 1. Hemodynamic parameters.

<table>
<thead>
<tr>
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<th>CTL</th>
<th>PPH</th>
<th>APH</th>
<th>APPH</th>
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</thead>
<tbody>
<tr>
<td>Systolic pressure (mmHg)</td>
<td>19.3±7.5</td>
<td>29.7±9.3(b)</td>
<td>27.9±7.4(b)</td>
<td>38.4±10.0(bcf)</td>
</tr>
<tr>
<td>Diastolic pressure (mmHg)</td>
<td>11.3±5.7</td>
<td>14.9±5.7(b)</td>
<td>13.8±6.3</td>
<td>17.7±7.1(af)</td>
</tr>
<tr>
<td>Mean pressure (mmHg)</td>
<td>14.0±6.2</td>
<td>19.8±6.7(b)</td>
<td>18.3±7.3(b)</td>
<td>24.7±7.6(bcf)</td>
</tr>
<tr>
<td>Pulse pressure (mmHg)</td>
<td>8.0±2.1</td>
<td>14.8±4.3(b)</td>
<td>14.1±3.3(b)</td>
<td>20.7±4.9(bcf)</td>
</tr>
<tr>
<td>Systolic diameter (mm)</td>
<td>23.8±2.3</td>
<td>24.7±2.1(b)</td>
<td>23.6±2.1(d)</td>
<td>24.4±2.1(af)</td>
</tr>
<tr>
<td>Diastolic diameter (mm)</td>
<td>22.6±2.4</td>
<td>23.6±2.2(b)</td>
<td>21.6±2.6(ad)</td>
<td>22.5±2.5(e)</td>
</tr>
<tr>
<td>Mean diameter (mm)</td>
<td>23.0±2.3</td>
<td>23.9±2.2(b)</td>
<td>22.3±2.4(ad)</td>
<td>23.2±2.3(e)</td>
</tr>
<tr>
<td>Pulse diameter (mm)</td>
<td>1.17±0.30</td>
<td>1.11±0.24</td>
<td>1.99±0.81(ac)</td>
<td>1.89±0.77(ac)</td>
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<tr>
<td>Heart rate (beats/min)</td>
<td>100±10</td>
<td>110±10</td>
<td>105±12</td>
<td>105±12</td>
</tr>
</tbody>
</table>

Values are mean±SD. (a)P<0.05 and (b)P<0.01, respect to CTL; (c)P<0.05 and (d)P<0.01, respect to PPH; (e)P<0.05 and (f)P<0.01, respect to APH.
Table 2. Incremental elastic modulus ($E_{inc}$) and some indexes used in clinical practice to wall mechanics characterization

<table>
<thead>
<tr>
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<th>CTL</th>
<th>PPH</th>
<th>APH</th>
<th>APPH</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{inc}$ ($10^6$ dyn/cm$^2$)</td>
<td>1.9±1.3</td>
<td>3.8±1.1 $^{(b)}$</td>
<td>1.7±0.9 $^{(d)}$</td>
<td>2.9±2.2</td>
</tr>
<tr>
<td>$P_{CS}$ (cm$^2$)</td>
<td>0.42±0.10</td>
<td>0.42±0.08</td>
<td>0.69±0.25 $^{(ac)}$</td>
<td>0.68±0.25 $^{(ac)}$</td>
</tr>
<tr>
<td>Normalized $P_{CS}$ (%)</td>
<td>10.8±3.6</td>
<td>9.8±2.7</td>
<td>20.3±10.6 $^{(ac)}$</td>
<td>18.2±9.3 $^{(a)}$</td>
</tr>
<tr>
<td>$D_{CS}$ (1/mmHg)</td>
<td>1.48±0.68</td>
<td>0.74±0.35 $^{(a)}$</td>
<td>1.50±0.75 $^{(c)}$</td>
<td>0.93±0.46 $^{(af)}$</td>
</tr>
<tr>
<td>$E_p$ ($10^5$ dyn/cm$^2$)</td>
<td>2.31±1.37</td>
<td>4.69±2.80 $^{(a)}$</td>
<td>2.54±1.76 $^{(c)}$</td>
<td>4.11±2.90 $^{(ae)}$</td>
</tr>
<tr>
<td>$C_{DY}$ (D/100 mmHg)</td>
<td>71.8±32.3</td>
<td>36.0±16.7 $^{(a)}$</td>
<td>71.1±34.8 $^{(c)}$</td>
<td>44.1±21.3 $^{(af)}$</td>
</tr>
</tbody>
</table>

Values are mean±SD. $P_{CS}$: cross sectional pulsatility. $D_{CS}$: cross-sectional distensibility. $E_p$: pressure-strain elastic modulus. $C_{DY}$: dynamic compliance. $^{(a)}P<0.05$ and $^{(b)}P<0.01$, respect to CTL; $^{(c)}P<0.05$ and $^{(d)}P<0.01$, respect to PPH; $^{(e)}P<0.05$ and $^{(f)}P<0.01$, respect to APH.
Table 3. Values for uncertainties of $E_P$, $C_{DYN}$ and $D_{CS}$ averaged during control state (CTL), and during passive pulmonary hypertension (PPH), active pulmonary hypertension (APH), and combination of both (APPH).

<table>
<thead>
<tr>
<th></th>
<th>$E_P$ uncertainty (% of the mean)</th>
<th>$C_{DYN}$ uncertainty (% of the mean)</th>
<th>$D_{CS}$ uncertainty (% of the mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTL</td>
<td>67.6 ± 9.0</td>
<td>49.8 ± 1.0</td>
<td>50.1 ± 0.9</td>
</tr>
<tr>
<td>PPH</td>
<td>68.9 ± 10.1</td>
<td>51.7 ± 1.6</td>
<td>51.6 ± 1.3</td>
</tr>
<tr>
<td>APH</td>
<td>76.0 ± 5.7</td>
<td>52.2 ± 0.4</td>
<td>53.5 ± 0.4</td>
</tr>
<tr>
<td>APPH</td>
<td>77.7 ± 6.8</td>
<td>51.6 ± 0.6</td>
<td>52.6 ± 0.5</td>
</tr>
</tbody>
</table>

Values are mean±SD. $E_P$: pressure-strain elastic modulus. $C_{DYN}$: dynamic compliance. $D_{CS}$: cross-sectional distensibility.
Figure 1. Surgical instrumentation of the main pulmonary artery (PA): D-D': piezoelectric diameter gauges; P: pressure microtransducer; F: perivascular flow probe. Occ: pneumatic occluder around the left branch of the PA.
Figure 2. Graphs showing a representative pressure-diameter loop obtained in each experimental condition in the six sheep under study. Note that activation of vascular smooth muscle by phenylephrine (active pulmonary hypertension, APH) induced in all animals an isobaric contraction in diameter respect to passive pulmonary hypertension (PPH). Note that mechanical occlusion during phenylephrine administration (APPH) determined in all animals an isometric condition respect the control state (CTL).
Figure 3. Arterial conduit (CF) and buffer (BF) function indexes assessed during control state (CTL), and during passive pulmonary hypertension (PPH), active pulmonary hypertension (APH), and combination of both (APPH). Passive hypertensive states were made via pulmonary artery cuff occlusion, and active hypertensive states were induced by intravenous infusion of phenylephrine. Isobaric (black columns) and isometric (white columns) conditions are indicate. $Z_C$: characteristic impedance. $E_{PD}$ and $\eta_{PD}$: elastic and viscous pressure-diameter index. Values are mean±SD. (a) $P<0.05$ and (b) $P<0.01$, respect to CTL; (c) $P<0.05$ (d) and $P<0.01$, respect to PPH; (e) $P<0.05$ and (f) $P<0.01$, respect to APH.