Korean J Physiol Pharmacol
Vol 3: 321-328, June, 1999

Characteristics of Hypoxic Pulmonary Vasoconstriction of the Rat: Study by the Vessel Size and Location in the Lung

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Pulmonary blood vessels with diameters of 200~400 μm produce considerably more force in response to vasoconstrictor drugs than those which are either smaller or larger. We have therefore investigated whether or not hypoxic pulmonary vasoconstriction (HPV) is more powerful in vessels of these diameters. We have also looked at the possibility that vessels from different regions of the lung respond differently. To do this we have grouped vessels according to their location within the lung as well as by size. We used a small vessel myograph (Cambustion AM10, Cambridge, UK) to study 208 preconstricted (1 μM $PGF_{2\alpha}$) small pulmonary arteries (300~800 μm diameter when stretched to a tension equivalent to 25 mmHg transmural pressure) from 39 rats anaesthetized with 2% inspired halothane. A biphasic contraction was observed in response to hypoxia (ca. 25 mmHg Po_2). The magnitudes of both the first, transient, phase (PT, peak tension) and of the second, sustained, phase (SST, steady state tension) were measured. The latter was measured 40 min after the start of hypoxia. The first phase was most pronounced in vessels with an average diameter of 423 μm while the second phase was most pronounced in larger vessels (mean diameter 505 μm). These maximal responses were all seen in vessels somewhat larger than reported by others. The responses of smaller vessels (400~500 μm) did not depend upon their location within the lung, but those of larger vessels (600~700 μm) showed regional differences. Those from the right lobe and those from the base of the lung gave the largest responses. It was especially noticeable that large vessels (631 μm diameter) from the base of the right lung gave the biggest responses. Thus HPV seems to occur not in a uniform manner, dependent solely to the size of vessels, but it also depends to some degree on the region of the lung from which vessels have been taken. Furthermore, our results suggest that larger vessels, as well as smaller ones, may contribute significantly to HPV.

Key Words: Hypoxic pulmonary vasoconstriction, Regional differences, Hypoxia, Vessel size

INTRODUCTION

Hypoxic pulmonary vasoconstriction (HPV) was first demonstrated by von Euler and Liljestrand in the cat (1946). It is the constriction of pulmonary blood vessels, predominantly small precapillary arteries, in response to alveolar hypoxia. It tends to sustain systemic arterial oxygen tension by shunting pulmonary arterial blood flow from less ventilated areas of the lung to better ventilated ones.

Although HPV was originally described in the cat, it has since been demonstrated in a wide variety of mammalian species. There is marked variation, however, in the strength of the response according to species (Peake et al, 1981; Elliot et al, 1991), age (Rendas et al, 1982; Tucker et al, 1982), and sex (Wetzel et al, 1984).

In addition the strength of the response depends upon vessel size. Small precapillary pulmonary arteries ranging in diameter from 200 to 800 μm appear to dominate the control of blood flow in the pulmonary circulation. In experiments on a wire myograph, isolated vessels with a diameter of 200~400

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μm have been reported to give more force in response to vasoconstrictors than either smaller (100~200 μm) or larger (400~2,000 μm) vessels (Leach et al, 1989). Angiograms have also shown that small pulmonary arteries (200~300 μm) give maximal reductions in diameter upon exposure to hypoxia (Shirai et al, 1986). However, very small vessel and large pulmonary artery also respond to hypoxia, though to a lesser degree. For example, experiments using a real-time confocal laser scanning luminescence microscope (Yamaguchi et al, 1998) and in micropuncture experiments on subpleural vessels (Nagasaka et al, 1984) hypoxia contracted very small precapillary arterioles (20~50 μm). Smooth muscle cells from small pulmonary arteries also contract more forcefully than cells taken from the main pulmonary artery (Madden et al, 1992).

The questions which we address here are (1) Do myograph experiments show that HPV is most pronounced in vessels within the 200~400 μm range of diameters seen in response to other vasoconstrictors? (2) Does HPV affect vessels of a wide range of diameters, or does it affect only a short segment around the diameter that gives maximal responses? (3) Do vessels of equal diameter, but from different parts of the lung behave similarly? To answer these questions, we used a myograph to determine and compare the characteristics of HPV shown by small pulmonary arteries of different diameters and from different parts of the lung.

METHODS

Preparations and vessel mounting

Male Wistar rats (250~350 g) were anaesthetized with 2% halothane in 100% oxygen flowing at a rate of 2 L/min. Once no withdrawal reflex was shown, a midline abdominal incision was made and 500 units of heparin were injected into the inferior vena cava. The anterior thoracic cage was removed and the heart and lungs were excised and placed on a Sylgard-lined dissecting dish. Normal physiological saline solution (PSS; 119 mM NaCl, 4.7 mM KCl, 1.18 mM KH_2PO_4, 1.17 mM $MgSO_4$, 25 mM $NaHCO_3$, 5.5 mM glucose, and 2 mM $CaCl_2$) was used to bathe the organs throughout the dissection. The lungs were stretched and pinned to the dish with the parietal pulmonary surface facing up. The arterial tree, which lay underneath the airways, was located and cleaned from the attached lung parenchyma. Vessels of 1.5~2.0 mm in length, from the third generation, were dissected out and transferred using forceps to the experimental chambers of the myograph.

Isometric tension recording

Isolated vessel experiments were carried out using an automated myograph (Cambustion AM10, Cambridge, UK). Up to six vessels could be mounted on 40 μm tungsten wires fixed to the jaws of the myograph. The myograph bath contained PSS adjusted to give a pH of 7.4 when bubbled with 5% CO_2. A personal computer (Dell 386) equipped with software supplied by Cambustion Limited was used to control the myograph jaws, to simulate a transmural pressure of 25 mmHg, and to record data points. Data was sampled at 1 Hz and stored on disk for later graphing and analysis.

Normalization

The normalization protocol (Mulvany & Halpern, 1977) involved the construction of a length-tension curve for each vessel by stretching it in small increments and measuring the tension developed at each increment. The computer calculated (from the Laplace relation) and plotted the 25 mmHg transmural pressure isobar on the screen, and the vessel was stretched slowly until its wall tension was equilibrated at a point on this line. This procedure was performed while gassing the bath with normoxic gas (21% O_2, 5% CO_2, 100 ml/min).

Experimental protocol

Once normalization was complete the vessels were allowed 30 min to adjust to resting conditions. After this the vessels were preconstricted with 1 μM $PGF_{2\alpha}$ for an additional 10 min - a treatment which greatly increases the responsiveness of vessels to hypoxia (Agrawal et al, 1992, Jin et al, 1992). The vessels were then exposed to a hypoxic challenge of 0.7% O_2 and 5% CO_2 (balance N_2), giving a bath Po_2 of ca. 30 mmHg. In most experiments 40 min hypoxic challenges were used.

Statistical analysis

Comparison of tension was made by the independent t test. Values were presented as mean± SEM, and $P<0.05$ was taken to be statistically significant.

RESULTS

Biphasic response of isolated pulmonary arteries to hypoxia

In each experiment vessels were removed from the apical, middle and basal regions of the lower lobe of either lung (Fig. 1A). All gave similar biphasic patterns of response to hypoxia (Fig. 1B). The first phase of this response began to appear within approximately 5 min of the start of a hypoxic challenge. This phase was large in amplitude but relatively short in duration. After approximately 15 min the vessel had returned to near its resting tension, and the slower, sustained second phase now became apparent. This second phase usually plateaued to a steady-state response about 40 min after hypoxia was induced. Fig. 1B demonstrates that the peak tension of the second phase was reached after about 40 min and that after this it was reasonably well sustained for up to 2 hr.

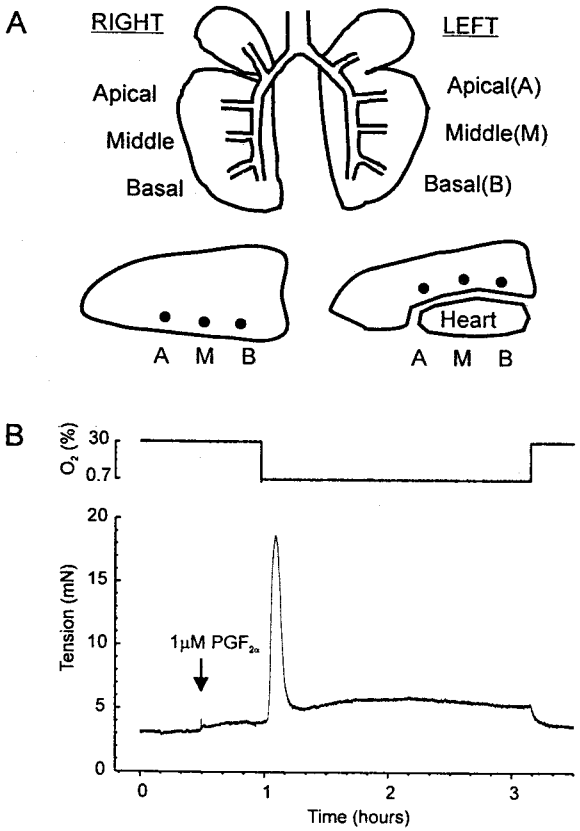

Fig. 1. Biphasic nature of hypoxic pulmonary vasoconstriction (HPV) response of the rat. A: All the vessels used in this experiment were third branches of pulmonary artery with internal diameter of 300~800 microns. They were classified into three groups (apical : A, middle : M and basal : B) by the region where the vessels were taken out. B: Representative HPV response. The maximum tension achieved by the second phase was held up to 130 minutes. Upon return to normoxic conditions, the vessels returned to their baseline tension.

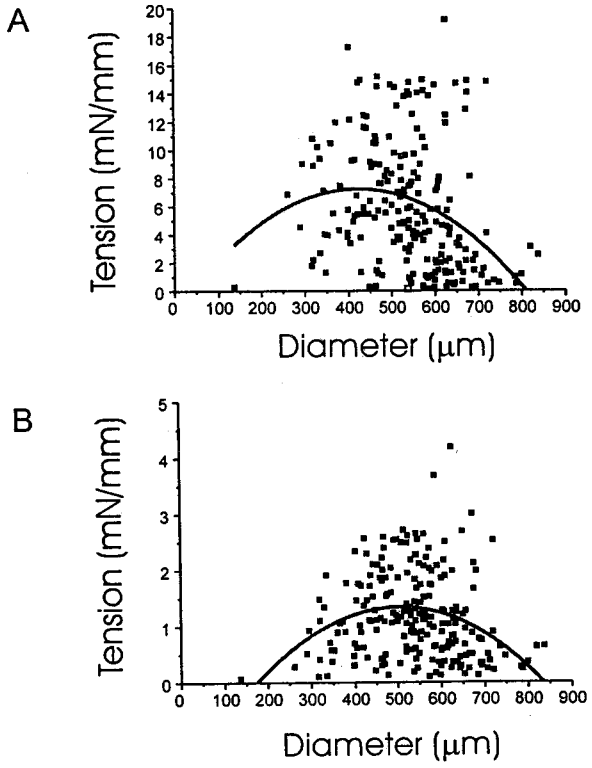

Fig. 2. Relationship between vessel diameter and maximal tension with fitted function. A: All the peak values of the first phase of HPV were drawn together with a fitted function of polynominal regression (order=2). According to the function vessel of 423 μm internal diameter seems to be best in producing the first phase. B: The graph of the second phase. It demonstrates vessels of around 505 μm internal diameter is better producing the second phase.

Table 1. Parameters and predicted peak tensions. All the raw data were fitted with a function of polynominal regression (order=2) in Fig. 1 and 2, and their parameters (a, b and c) used were shown with peak values calculated. Function: $y=a+bx+cx^2$

Vessel		Parameter			Peak	
		a	b	c	x (μm)	y (mN/mm)
Whole	PT	−1.475510	0.041130	−0.000049	423	7.23
	SST	−1.793100	0.012410	−0.000012	505	1.34
Left	Apical	−1.483280	0.010610	−0.000011	472	1.02
	Middle	−1.490720	0.011780	−0.000012	474	1.30
	Basal	−4.607730	0.024790	−0.000025	493	1.51
Right	Apical	−3.299430	0.018750	−0.000019	483	1.23
	Middle	−1.132180	0.010390	−0.000010	500	1.46
	Basal	−6.002410	0.025130	−0.000020	631	1.92

Table 2. Comparison between the left and right lobes of averaged tension classified to vessel size and location. Independent *t* test was done. All values are means±SEM, Abbreviation: NS, not significant; S.E., standard error; PT, peak tension (the first phase); SST, steady-state tension (the second phase).

Vessels		PT (mN/mm)			SST (mN/mm)		
Diameter	Location	Left	Right	P value	Left	Right	P value
400~499 μm	apical	5.1±1.6 (n=8)	8.3±2.1 (n=6)	NS	1.1±0.3 (n=8)	1.4±0.3 (n=6)	NS
	middle	8.8±1.3 (n=6)	8.9±1.5 (n=9)	NS	1.4±0.3 (n=6)	1.6±0.2 (n=9)	NS
	basal	8.9±1.3 (n=14)	7.0±0.9 (n=5)	NS	1.5±0.2 (n=14)	1.4±0.2 (n=5)	NS
500~599 μm	apical	4.0±1.1 (n=7)	4.4±0.8 (n=16)	NS	1.1±0.2 (n=7)	1.0±0.2 (n=16)	NS
	middle	5.6±1.3 (n=12)	6.0±1.0 (n=18)	NS	1.4±0.3 (n=12)	1.4±0.1 (n=18)	NS
	basal	7.0±1.1 (n=11)	10.2±1.1 (n=13)	NS	1.5±0.2 (n=11)	1.7±0.2 (n=13)	NS
600~699 μm	apical	1.3±0.2 (n=10)	3.7±0.8 (n=11)	<0.05	0.5±0.1 (n=10)	0.9±0.2 (n=11)	NS
	middle	1.8±0.4 (n=11)	4.9±1.3 (n=8)	<0.05	0.6±0.1 (n=11)	1.3±0.2 (n=8)	<0.01
	basal	3.0±1.7 (n=2)	10.8±1.5 (n=12)	NS	0.8±0.3 (n=2)	1.9±0.3 (n=12)	NS

Relationship between vessel diameter and peak tension

The first and the second phases of the hypoxic response were compared separately. All peak values (n=208) of the first and the second phases are shown in Fig. 2A and B respectively. When fitted with a quadratic function peak values were estimated to be 423 μm, 7.23 mN/mm for the first phase and 505 μm, 1.34 mN/mm for the second phase (Table 1). The second phase could be distributed among the 6 regions (Fig. 3). Most of the estimated maximal tension points were very similar, 1 to 1.5 mN/mm for vessel diameters slightly below 500 μm. However, vessels from the base of the right lung peaked at 1.92 mN/mm with vessel diameters of 631 μm (Table 1). When the first phase was distributed among the 6 regions, estimation of peak value was impossible in some regions because there were too few data points in the range of small diameter vessels to predict a peak value using a quadratic function.

Comparison between vessels of the right and the left lobes of the lung

We compared the responses of the first and the second phase between the right and the left lobes at approximately the same levels within the lung (Fig.

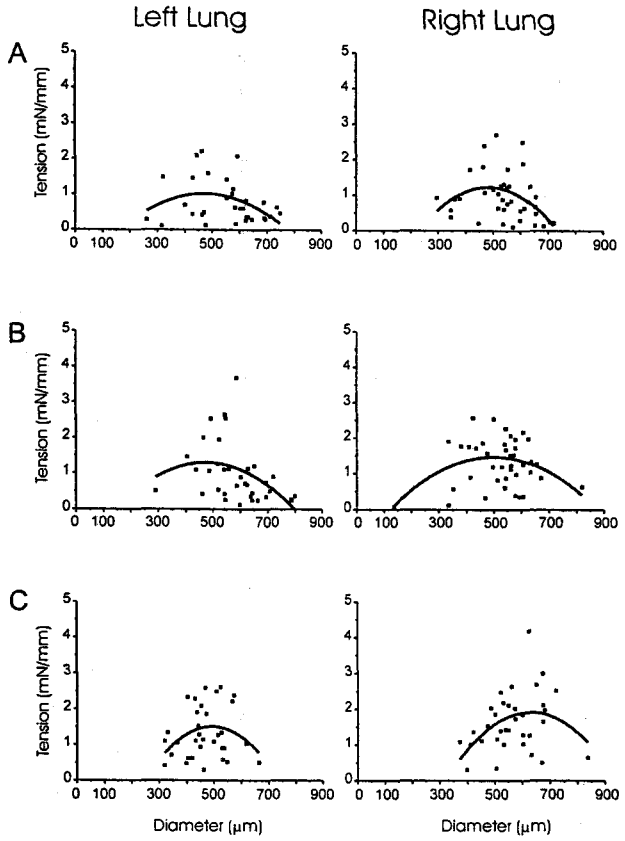

Fig. 3. Comparison of diameters producing steady state peak tension of the second phase with fitted function. The second phase responses were compared in the three regions; Apical (A), Middle (B) and Basal (C). The best response appeared to occur near vessels of 500 microns diameter except for right lower region vessels, which showed peak value near 600 microns.

1A) and also in vessels of similar diameter (Table 2). The responses of the smallest vessels (400~499 μm diameter) within each of the three regions, did not depend upon the lung which they had been taken from. The medium sized vessels (500~599 μm) from the right lung appeared to contract more forcefully, but this was not significant. However, the largest vessels (600~699 μm) from the right lung did contract more forcefully than those from the left lung. However, as can be seen in Table 2, the largest vessels (600~699 μm) from the base of the right lung did not contract with a significantly greater force than those from the left. This may be because here there were only two vessels from the left lung.

DISCUSSION

Features of HPV

In this study, the contractions observed in response to hypoxia in small pulmonary arteries were biphasic. The first phase is thought to be due mainly to the release and re-uptake of Ca by intracellular stores while the second phase is due to the influx of Ca through voltage-activated Ca channels (Albarwani et al, 1994). The first phase generally appeared within 5 minutes of the onset of hypoxia and lasted about 10 min. The slowly-developing second phase then became visible, and reached a steady state approximately 25 min. later. This second phase was maintained for 2 hr in our experiment (Fig. 1B) and would presumably have been maintained for even longer.

While in most recent work pulmonary arteries contract biphasically in response to hypoxia (Bennie et al, 1991; Agrawal et al, 1992; Jin et al, 1992), some researchers have reported that their preparations do not show a biphasic response (Rodman et al, 1990; Yuan et al, 1990). In addition, some researchers define the various phases of the *in vitro* response to hypoxia differently. Whereas we and others (Bennie et al, 1991; Albarwani et al, 1992; Jin et al, 1992; Robertson et al, 1993) consider the transient rise and subsequent relaxation to be the first phase, and the slow rise which follows to be the second phase, some have observed a biphasic response but used the term "second phase" for the relaxation towards baseline tension (Ogawa et al, 1993). Yet others have counted as many as four different phases of contraction and relaxation (Teng & Barer, 1995).

The second phase of the response resembles the slow monophasic contraction of the isolated lung both in shape and in duration; thus it may serve a more important role *in vivo* in HPV than the transient and more irregular first phase (Vejlstrup & Dorrington, 1993). Furthermore, the fact that the first phase can be seen in systemic as well as in pulmonary vessels (Muramatsu et al, 1992) indicates that it is not exclusively a characteristic of HPV. Our results give some support to this, since the different optimal diameters for maximal responses during the first and the second phases might be interpreted to suggest that they have different causes (Fig. 2).

Size of pulmonary artery and HPV

In our experiments the most forceful first phase contractions were obtained from vessels with diameters of about 423 μm. For the second phase the most forceful contractions were obtained from larger vessels. In most regions the latter were about 500 μ m in diameter, but in vessels from the base of the right lung the most forceful contractions were obtained for those of a diameter of about 631 μm (Table 1). Thereafter the force of contraction declined as vessel diameter increased.

All these results are consistent with the finding that smooth muscle cells from main pulmonary arteries contract less than those taken from small pulmonary arteries (Madden et al, 1992). The optimal range in our experiments was larger than the 200~300 μm range given for the maximal contraction of small pulmonary arteries in angiograms of hypoxic vessels (Shirai et al, 1986). This could be due to the fact that, in myograph experiments, the smaller arteries (200~300 μm) are easily damaged as they are being mounted on the 40 μm wires. However, because significantly larger diameter vessels gave more forceful contractions in the second phase than that in the first phase, it appears that damage may not account for all of the loss of strength as vessel diameter is reduced. Since the second phase of contraction in isolated vessels is probably more important than the first phase in the generation of HPV, our finding that larger vessels respond more forcefully in this phase suggests that these may play a more important role than has previously been assumed.

Physiological relevance of regional difference of HPV

Unlike other vascular beds, which are perfused by the left heart in parallel with each other, the pulmonary circulation forms the only route from the right to the left heart, i.e. it is in series with all other tissues. Thus it cannot adjust its own overall flow without also affecting the flow to the rest of the body. The pulmonary circulation is therefore obliged to accommodate all the cardiac output. In order to do this, and to ensure that the blood is oxygenated in the most efficient fashion, the lungs rely on HPV to modify regional intrapulmonary blood flow. As a result, large volumes of blood flow through regions of high oxygen tension and only small volumes through regions where oxygen tension is low. In general the smaller the hypoxic area, the greater the percentage of blood can divert from hypoxic region and the smaller will be the resulting rise in pulmonary arterial pressure (Marshall & Marshall, 1980). When hypoxia is global, as in the case of residents of high altitudes, the shunting of blood flow becomes impossible. However, as long as this hypoxia is moderate, HPV is still successful in enhancing pulmonary gas exchange in human and other large upright animals because it increases perfusion to the apices of the lung (Cutaia & Rounds, 1990). Therefore the overall ventilation-perfusion ratio is improved. In contrast, small animals like rats do not experience significant gravitational influences simply because they are too small. However, our experimental data suggests a possible existence of regional differences. Since the rat is normally in the prone position, the dorsal region of the lung becomes upper region and the ventral region becomes lower region. Since the heart occupies most of the basal region on the left side (Fig. 1A) there is a major difference between the two lungs. This means that there is no lower region in the left lung equivalent to the counterpart of the right lung. The right basal region appears to be the only part low enough to be able to show maximal gravitational influence in the rat. In accordance with this expectation large vessels of right lung showed more forceful HPV response than left ones (Table 2). However, since the gravitational difference of both lung is extremely small there are other possibilities to explain this results. They might be explained by something to do with the position of the heart - perhaps the heart's presence or activity affecting the resistance or impedance of flow to the area around it. We could not explain the regional difference of the right lower vessels, which, indeed remarkably, showed biggest response and had a peak response in the diameter of 631 μm.

Our findings further showed that relatively large vessels (600~700 μm) contract most forcefully in response to hypoxia, particularly in right lower lobe. Smaller vessels (400~500 μm) behaved similarly, and contracted less forcefully, in all the regions examined. This result contrasts with the results of Shirai et al who found that much smaller vessels contracted most. However it would be inappropriate to compare our results directly with theirs because they applied hypoxia for only 7~9 min, a period corresponding to the first phase. Furthermore they used only the left

lower lobes; a region in which we found only small effects.

In conclusion, we have shown that, in isolated vessels on a myograph, HPV seems to vary according to location within the lung as well as according to vessel diameter. We have also shown that the second, sustained, phase of contraction is most forcefully expressed by vessels of a larger diameter than has been previously supposed.

ACKNOWLEDGEMENT

The authors wish to acknowledge the financial support of the Korea Research Foundation made in the program year of 1997. We would like to thank Dr. Piers Nye (University Laboratory of Physiology, Oxford University, U.K.) for useful discussions and helping with English, and Mr. Chris Hirst for giving technical support to this experiment. This work was also funded in part by the Wellcome Trust.

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