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# Acute Hypoxic Vasoconstriction in Isolated Rat Small and Large Pulmonary Arteries

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## Summary

Hypoxic vasoconstriction (HPV) has been shown to consist of a biphasic contraction change. The first phase of the hypoxic response peaks at approximately five minutes. The second phase is at about 30 minutes. The force of contraction of both phases of HPV were found to be significantly greater in pulmonary resistance vessels (PRV) than in pulmonary artery (PA) ($P<0.01$). The endothelium modulates the hypoxic response, especially of the second phase of HPV (68 % reduction in PRV) ($P<0.05$). In $Ca^{2+}$-free solution, the first peak and the second peak of HPV were reduced to 11 and 32 % contraction in PRV and to 26 and 21 % contraction in PA. A calcium channel antagonist (amlodipine) caused significant dose-dependent inhibition of the first phase of HPV ($P=0.001$), with a significantly greater effect on PRV compared to PA ($P<0.01$). Levcromakalim caused a dose-dependent inhibition of HPV in PRV (58 % at 10 $\mu$M). In contrast, HPV in PA was not significantly inhibited by levcromakalim. In conclusion, this study has confirmed that hypoxia induces a biphasic contractile response in isolated pulmonary arteries requiring extracellular calcium. Both amlodipine and levcromakalim inhibit hypoxic pulmonary vasoconstriction and these agents may be of value in the treatment of pulmonary hypertension.

**Key words**

Hypoxic pulmonary vasoconstriction – Pulmonary artery – Pulmonary resistance vessels

## Introduction

The vasoconstriction response to hypoxia is a unique feature of pulmonary circulation. This effect was first described by von Euler and Liljestrand (1946). In a rat ventilated with a gas mixture of 10 to 11 % $O_2$ in $N_2$ an increase in pulmonary arterial pressure was observed. This protective reflex ensures correct matching of ventilation and perfusion and is thought to act through membrane depolarisation secondary to alteration of potassium flux in pulmonary vascular smooth muscle (Fishman 1976). In the lung, resistance arteries are larger than 50 $\mu$m (Bhattacharya *et al.* 1982) and the principal site of hypoxic vasoconstriction is believed to be arteries with internal diameter between 200 and 300 $\mu$m (Rodman and Voelkel 1991). However, the finding by Madden *et al.* (1985) suggested that the large pulmonary arteries (>500 $\mu$m) exhibited a similar but smaller hypoxic contraction.

The role of the pulmonary endothelium, which is to sense hypoxia, was investigated by both De Mey and Vanhoutte (1982) and Holden and McCall (1984). Both studies concluded that the endothelium was acting as an hypoxic sensor. However, Burke and Wolin (1987) observed hypoxic contraction in endothelium-denuded bovine pulmonary artery rings and concluded that pulmonary artery smooth muscle cells also sense hypoxia.

Recently, Farrukh and Michael (1992) suggested that hypoxic pulmonary vasoconstriction directly correlates with extracellular calcium ($[Ca^{2+}]_o$). The absence of $[Ca^{2+}]_o$ in the perfusate markedly attenuates the hypoxaemia-induced pulmonary vasoconstriction in isolated blood-perfused ferret lungs. Albarwani *et al.* (1994) also found the complete failure of the first phase of hypoxic pulmonary vasoconstriction when $[Ca^{2+}]_o$ is removed, suggesting that the first phase is triggered by the influx of $[Ca^{2+}]_o$. McMurtry *et al.* (1976) reported that the calcium antagonist verapamil was effective in inhibiting hypoxic pulmonary vasoconstriction (HPV) in isolated perfused rat lungs. Numerous studies with various calcium antagonists have confirmed this effect in isolated lungs (Kennedy and Summer 1982), whole animals (Young *et*

*al.* 1983, Dickstein *et al.* 1984, Archer *et al.* 1985) and humans (Naeije *et al.* 1982, Brown *et al.* 1983, Kennedy *et al.* 1984). It is only relatively recently that small (Rodman and Voelkel 1991) and large (Rodman *et al.* 1989) pulmonary arteries have been studied in isolation. However, HPV has been observed in isolated pulmonary artery specimens of around 2–3 mm diameter from rats (Rodman *et al.* 1989) and humans (Ohe *et al.* 1992). In both cases, HPV was inhibited by calcium antagonists.

The alteration of ventilation-perfusion relationships may be important in disease states and in chronic obstructive pulmonary disease can lead to an increase in pulmonary vascular resistance, resulting in pulmonary hypertension and eventual right heart failure. The use of conventional vasodilators in this condition is controversial and has been confounded by alteration of ventilation-perfusion matching leading to a fall in oxygen saturation. At present, treatment is limited to long-term oxygen therapy, which is inconvenient for the patient (Medical Research Council Working Party 1981). Cromakalim is a novel relaxant of smooth muscle which is believed to act by enhancing potassium efflux from the cell and thereby hyperpolarising the plasma membrane (Hamilton and Weston 1989, Quast and Cook 1989). The effects of cromakalim are stereospecific and confined to the (−)-enantiomer (levcromakalim) (Buckingham *et al.* 1986, Hof *et al.* 1988). Cromakalim is a potent anti-hypertensive agent in both laboratory animals (Buckingham *et al.* 1986) and man (Vanden Burg *et al.* 1987).

Amlopidine is a recently introduced dihydropyridine $Ca^{2+}$ antagonist with a slow onset of action and a half-life of around 35–40 hours in healthy humans. The reason for this delayed onset of action is unknown. Nevertheless, it has been suggested that the dihydropyridine receptor site on the voltage-sensitive calcium channel is located in a hydrophobic region (Catterall and Striessnig 1992) and that dihydropyridine calcium antagonists must diffuse through the lipid bilayer of the cell membrane for binding (Lucchesi 1989). Unlike other dihydropyridines, which are lipophilic, amlodipine is more than 90 % ionized at physiological pH. It is therefore possible that the rate-limiting step for amlodipine receptor binding is due to its slow diffusion into the lipid bilayer resulting in the unusually slow tissue kinetics (Burges *et al.* 1987, 1989). This unusual pharmacokinetic profile appears to reduce the severity of side effects (Murdoch and Heel 1991) suggesting that amlodipine might be a valuable agent in the treatment of primary pulmonary hypertension. However, very little is known about the action of amlodipine in the pulmonary circulation.

In this investigation, the purpose was to determine if rat pulmonary artery contracted in response to hypoxia and to examine how extracellular calcium, amlodipine, levcromakalim and removal of the endothelium affected the hypoxic response in isolated small and large pulmonary arteries.

## Methods

*Tissue preparation*

Adult male Wistar rats (250–300 g) were anaesthetized by intraperitoneal injection of pentobarbitone sodium (15 mg/100 g body weight). The chest was opened, the heart and lungs were removed and placed in cold physiological saline solution (PSS). Using an operating microscope to obtain pulmonary arterial specimens the left lung was pinned to a Petri dish with the visceral side exposed. The broncho-vascular bundles were then identified and the bronchi dissected away from the underlying artery or arteriole. Rings of pulmonary vessels were dissected free from surrounding connective tissue and removed.

*Measurement of vasoreactivity*

All experiments reported were performed on a computer-controlled automated myograph (Cambustion Ltd., Cambridge, UK). The technique has been described in full elsewhere (Mulvany and Halpern 1977, Rogers *et al.* 1992). Briefly, each organ bath of the myograph accommodated two vessel segments, each one mounted on a pair of jaws as a ring preparation. Vessels were mounted in the following way. Two nonelastic tungsten wires (diameter 40 $\mu$m) were passed longitudinally through the lumen. Each wire was fixed to opposing steel jaws. One jaw was attached to a drive motor and micrometer allowing control of movement and measurement of distance between the wires. The other jaw was connected to a force transducer so that a measure of vessel wall tension could be made. The force transducer was calibrated weekly against known weights.

In each experiment, a small and large artery was mounted in each organ bath. Vessels were bathed in PSS perfused with 95 % oxygen/5 % carbon dioxide and warmed to 37 °C. With the aid of the myograph software, the relationship between length and tension was plotted for each vessel. Software control automatically pretensioned vessels to simulate the required resting transmural pressure. Vessel diameter was derived from the vessel's circumference at a given resting pressure.

*Experimental protocol*

In all series of experiments vessels were pretensioned to 100 % of an equivalent internal pressure of 17.5 mm Hg. This resting tension was selected to approximate the pressure in pulmonary

arteries (Herget *et al.* 1978) and arterioles (Bhattacharya *et al.* 1982) of normoxic animals *in vivo*. Maximal KCl contractions were produced with 100 mM KCl. After 15 min the tensions were noted. The vessels were washed thoroughly with PSS and then allowed to relax fully. After the second loading procedure all experiments were conducted isometrically. Values quoted for vessel diameter are recorded after the second vessel loading. Then maximal KCl contractions were repeated twice more to ensure consistent results and viability of vessels.

*Response to hypoxia*

Precontracted rings were treated with the $PGF_{2\alpha}$ (6 $\mu$M), and the response to hypoxia was measured after steady-state tension was achieved. The induction of basal tone with $PGF_{2\alpha}$ was found greatly to enhance HPV. $PGF_{2\alpha}$ was used for this experiment because it produced a sustained and more reproducible precontraction than other agents tested (KCl, angiotensin II). The organ bath was sealed and perfused with 5 % carbon dioxide and 95 % nitrogen (the hypoxic gas). With the bath uncovered, this produced a resting $PaO_2$ within the PSS of 10–12 kPa. When the organ bath was sealed the $PaO_2$ decreased to 2–3 kPa. Perfusion was maintained until a maximum contractile response was seen, generally within 2–6 min (first peak of contraction). After 30 min a second peak was observed. Hypoxic responses are reported as peak force generated during the early and late phase of hypoxia. In some experiments, the effect of repeated hypoxia in small and large pulmonary arteries was studied.

Endothelium-free PRV rings were prepared by gently rubbing of the internal surface with a human hair. The removal of endothelium was confirmed by the reduction of relaxation by acetylcholine ($10^{-5}$ M) precontracted with $PGF_{2\alpha}$ ($10^{-4}$ M). Hypoxic responses were obtained in PRV rings with intact endothelium, then after rubbing of the internal surface, HPVs were repeated on endothelium-free PRV rings.

In other eight experiments, small and large arteries were mounted in the same organ bath. Vessels were bathed in $Ca^{2+}$ free PSS and warmed to 37 °C for at least one hour. Precontracted rings were treated with the $PGF_{2\alpha}$ (6 $\mu$M), and the response to hypoxia was measured after steady-state tension was achieved. The organ bath was sealed and perfused with 5 % carbon dioxide and 95 % nitrogen (the hypoxic gas) until hypoxic contractile response (both phases) was seen. Vessels were washed with a $Ca^{2+}$-containing PSS and one hour was allowed for equilibration. Hypoxic pulmonary vasoconstriction was then repeated in $Ca^{2+}$-containing PSS.

*Effect of levcromakalim on hypoxic pulmonary vasoconstriction*

To normalize the gas tension within the organ bath the cover was removed and the pulmonary arteries relaxed. After adding levcromakalim (1, 10, 100 $\mu$M), hypoxic response experiments were repeated in eight PA and PRV. Saline was added to control vessels.

*Effect of amlodipine on hypoxic pulmonary vasoconstriction*

In a previous study (Woodmansey *et al.* 1993), we have shown that amlodipine has an unusually slow onset of action and its effect at tissue level persists for several hours. The effect of the $Ca^{2+}$ antagonists persisted in the vessels after several washing periods. For this reason, the vessel segments were used only once. The first hypoxic challenge in each vessel was carried out as a control. To normalize the gas tension within the organ bath, the cover was removed and the pulmonary arteries relaxed. After adding amlodipine (0.1, 1, 10 $\mu$M) hypoxic response experiments were repeated in PA (n=32) and PRV (n=25). Saline was added to control vessel.

*Solution and drugs*

PSS consisted of (in mM): 120 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.17 $MgSO_4$, 25 $NaHCO_3$, 1.18 $KH_2PO_4$, 5.5 glucose and 26.9 $\mu$M EDTA dissolved in deionized water. The solution was continuously bubbled with a 95 % oxygen/5 % carbon dioxide mixture. $PGF_{2\alpha}$ was obtained from Sigma (Poole, Dorset, UK) and was dissolved in saline (150 mM NaCl). Levcromakalim (BRL 38227) a gift from Smith Kline Beecham Research Laboratories (The Pinnacles, UK) was dissolved in 50 % polyethylene glycol (PEG). The maximal concentration of PEG in the bath had no effect on the contractility of pulmonary artery in the rat. Amlodipine (Pfizer UK Ltd.) was also dissolved in 150 mM NaCl solution. Unlike nifedipine, amlodipine is not light sensitive, and is freely water soluble. All of the stock solutions were kept at −20 °C.

*Statistical analysis*

The amplitude of contraction is expressed as means ± S.E.M. Significance was assessed by Student's t-test. A difference between means was considered significant when $P<0.05$.

## Results

*Hypoxic pulmonary vasoconstriction in PRV and PA*

Precontraction with $PGF_{2\alpha}$ (6 $\mu$M) produced stable plateau responses in the large and small arteries. Pulmonary vasoconstriction also occurred in both large vessels and small vessels (Table 1). Hypoxic contraction was recorded at 5 min (first peak) and at 30 min (second peak) (Fig. 1). The hypoxic contraction was greater in PRV than in PA ($P<0.01$) (Fig. 2, Table 1)

**Table 1**
Hypoxic pulmonary vasoconstriction in pulmonary resistance vessels (PRV) and pulmonary artery (PA)

| | PRV | PA |
|---|---|---|
| n | 37 | 42 |
| Diameter ($\mu$m) | 245±9 | 992±36 |
| First peak (mN/mm) | 2.1±0.3** | 0.4±0.03 |
| Second peak (mN/mm) | 0.6±0.2* | 0.2±0.03 |

*Data representing active tension are expressed as means ± S.E.M.*
** $P<0.05$, ** $P<0.01$, (compared with PA)*

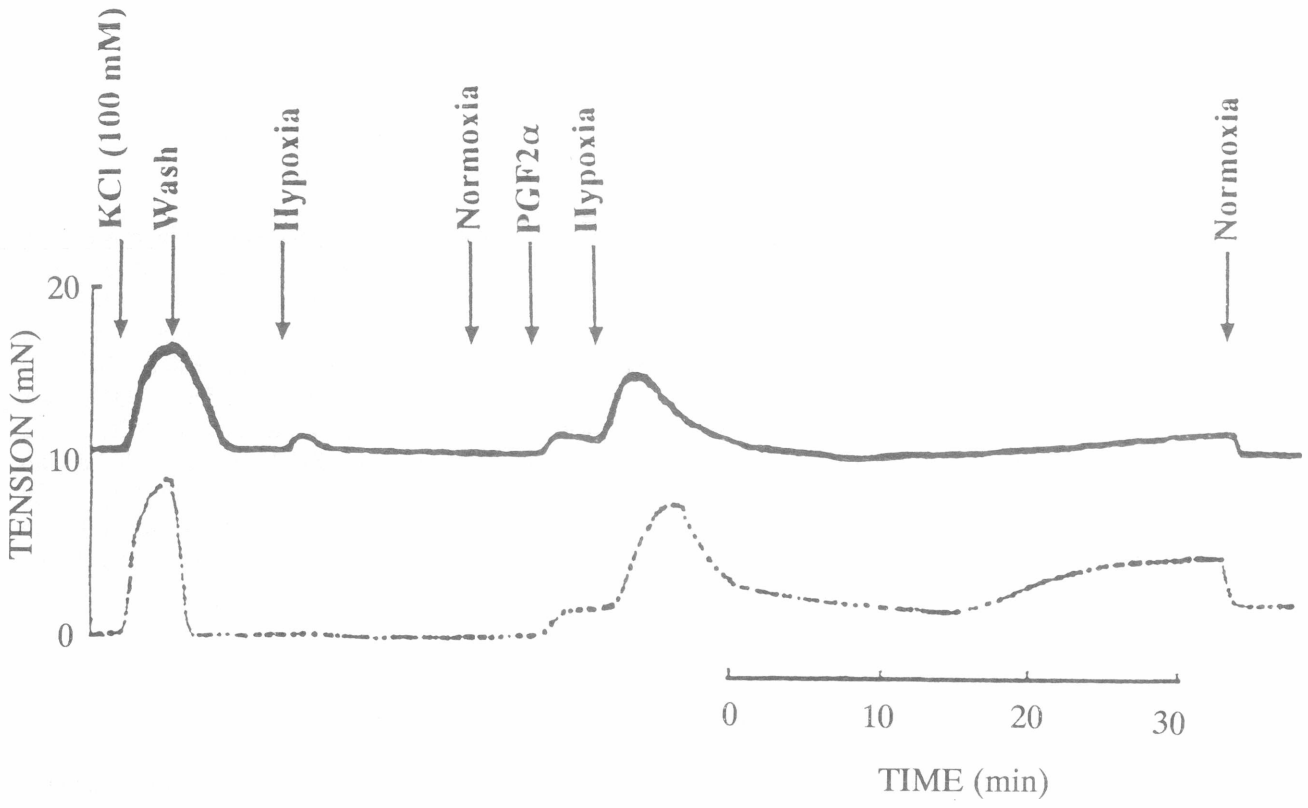


**Fig. 1**
Typical contraction induced by hypoxia (5 % $CO_2$ in $N_2$) in PA (full line) and PRV (broken line). Precontraction with $PGF_{2\alpha}$ (6 $\mu$M) produced stable plateau responses in both PA and PRV. Hypoxic contractions were recorded at 5 min (first peak) and at 30 min (second peak).

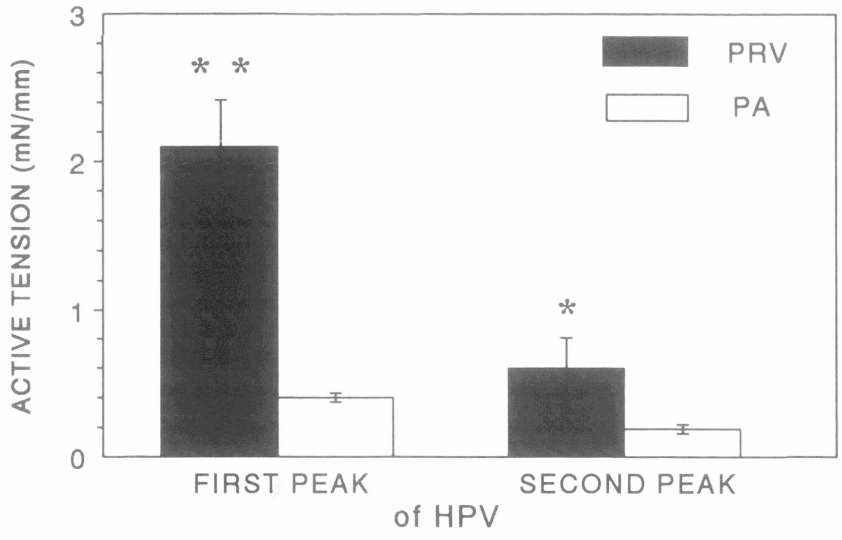


**Fig. 2**
Hypoxic responses are reported as peak force (active tension, mN/mm) generated during the early (first peak, 5 min) and late phases (second peak, 30 min) of hypoxia in isolated PRV and PA. * $P<0.05$, ** $P<0.01$, compared with PA.

**Table 2**
Contraction during the second hypoxic challenge in isolated large arteries and small resistance vessels

| Resistance vessels | | Large arteries | |
|---|---|---|---|
| First peak of HPV (n = 10) | Second peak of HPV (n = 9) | First peak of HPV (n = 12) | Second peak of HPV (n = 9) |
| 97.6 ± 1 | 190.8 ± 3 | 101.8 ± 1 | 152.2 ± 3 |

*Data in percentage changes from baseline are expressed as means ± S.E.M.*

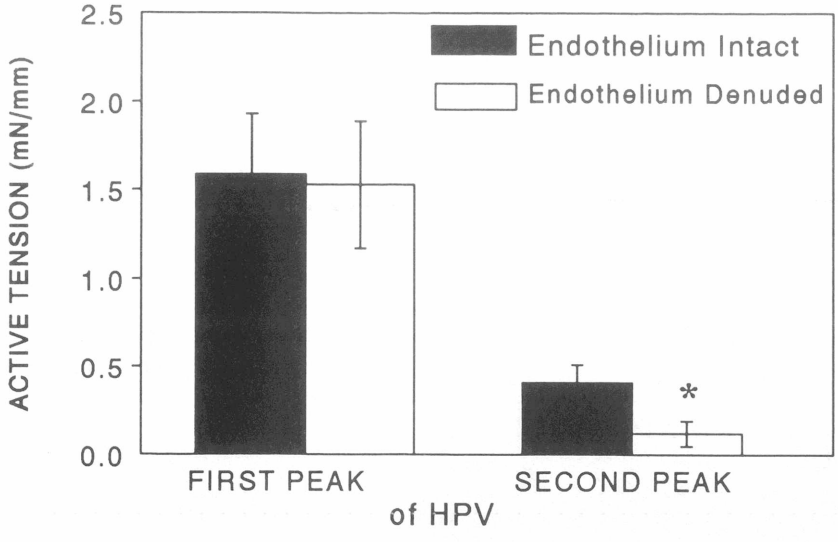


**Fig. 3**
Effect of removal of the endothelium on the hypoxic response in isolated precontracted rat PRV rings treated with $PGF_{2\alpha}$ (6 $\mu$M). Hypoxic response was measured in twelve pairs of PRV rings with intact or denuded endothelium. * $P<0.05$, compared with intact endothelium PRV.

*Effect of repeated hypoxia on small and large pulmonary arteries*

Table 2 shows that the first phase of HPV was highly reproducible within each vessel size, and there was relatively little variation between vessels. In contrast, the late phase during the second exposure to hypoxia was greater than on the first occasion in both vessel sizes, with great variability between vessels.

*Effect of endothelium removal on hypoxic contraction*

Removal of the endothelium reduced $10^{-5}$ M acetylcholine relaxation of twelve PRV rings (diameter $246 \pm 16$ $\mu$m, n=12) precontracted with $10^{-4}$ M $PGF_{2\alpha}$ from $54.2 \pm 2$ % to $7.6 \pm 1.5$ % ($P<0.001$). This result confirmed highly significant denudation of the endothelium. Fig. 3 shows the first peak and the second peak of HPV from twelve pairs of PRV rings with intact or denuded endothelium. The first phase of the hypoxic response in endothelium-denuded PRV rings is not significantly different compared to rings with intact endothelium ($P>0.05$). Nevertheless, the second phase of the hypoxic response in endothelium-denuded PRV rings demonstrated a 68 % reduction compared to PRV with intact endothelium ($P<0.05$).

*Hypoxic pulmonary vasoconstriction in $Ca^{2+}$-free solution*

Fig. 4 (upper panel) shows the effect of $[Ca^{2+}]_o$ in the first peak and the second peak of HPV in PRV. In the $Ca^{2+}$-free solution, the first peak and the second peak of HPV were reduced to 11 and 32 % contraction seen in $Ca^{2+}$-containing solution (Table 3). Fig. 4 (lower panel) shows the effect of $[Ca^{2+}]_o$ in the first peak and the second peak of HPV in PA. In the $Ca^{2+}$-free solution, the first peak and the second peak of HPV were reduced to 26 % and 21 % contraction occurring in $Ca^{2+}$-containing solution (Table 3).

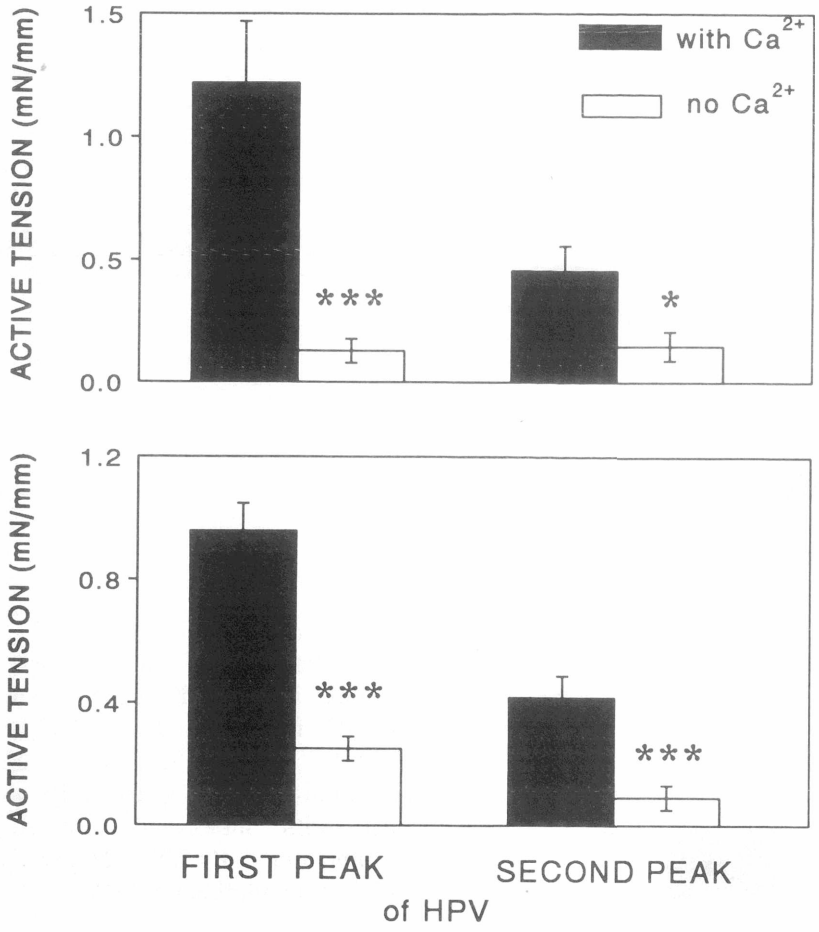


**Fig. 4**
The effect of $[Ca^{2+}]_o$ in the first peak and the second peak of HPV in PRV (upper panel) and PA (lower panel).

**Table 3**
Effect of $[Ca^{2+}]_o$ on HPV in isolated rat PRV and PA

| | PRV | | PA | |
|---|---|---|---|---|
| | $[Ca^{2+}]_o$-free | $[Ca^{2+}]_o$-containing | $[Ca^{2+}]_o$-free | $[Ca^{2+}]_o$-containing |
| Diameter ($\mu$m) | 273±13 | | 1023±32 | |
| First peak (mN/mm) | 0.13±0.07*** | 1.22±0.25 | 0.25±0.04*** | 0.96±0.09 |
| Second peak (mN/mm) | 0.15±0.06* | 0.46±0.11 | 0.09±0.05*** | 0.43±0.07 |

*Data representing active tension are expressed as means ±S.E.M. (n = 8).*
** P<0.05, *** P<0.001, compared with HPV in $[Ca^{2+}]_o$-containing PSS*

*Effect of Levcromakalim on hypoxia-induced contractions*

Fig. 5 shows a dose-dependent inhibition of the first peak and the second peak of HPV by levcromakalim (1, 10, 100 $\mu$M) in isolated rat PRV and PA. At the first peak of HPV a low concentration levcromakalim (1 $\mu$M) caused 29 % inhibition in PRV and 38 % inhibition in PA. The difference was not, however, significant. Higher concentrations of levcromakalim (10 and 100 $\mu$M) caused 58 % and 88 % inhibition, respectively, in PRV ($P<0.05$, $P<0.01$, compared with the baseline) and 61 % and 80 % inhibition, respectively, in PA ($P<0.05$, $P<0.05$). PRV had a significantly greater inhibition of HPV compared with PA at the highest concentration of levcromakalim (100 $\mu$M) ($P<0.05$). At the second peak of HPV, only the high concentration of levcromakalim (100 $\mu$M) caused a significant inhibition both in PA and PRV ($P<0.05$) (Table 4).

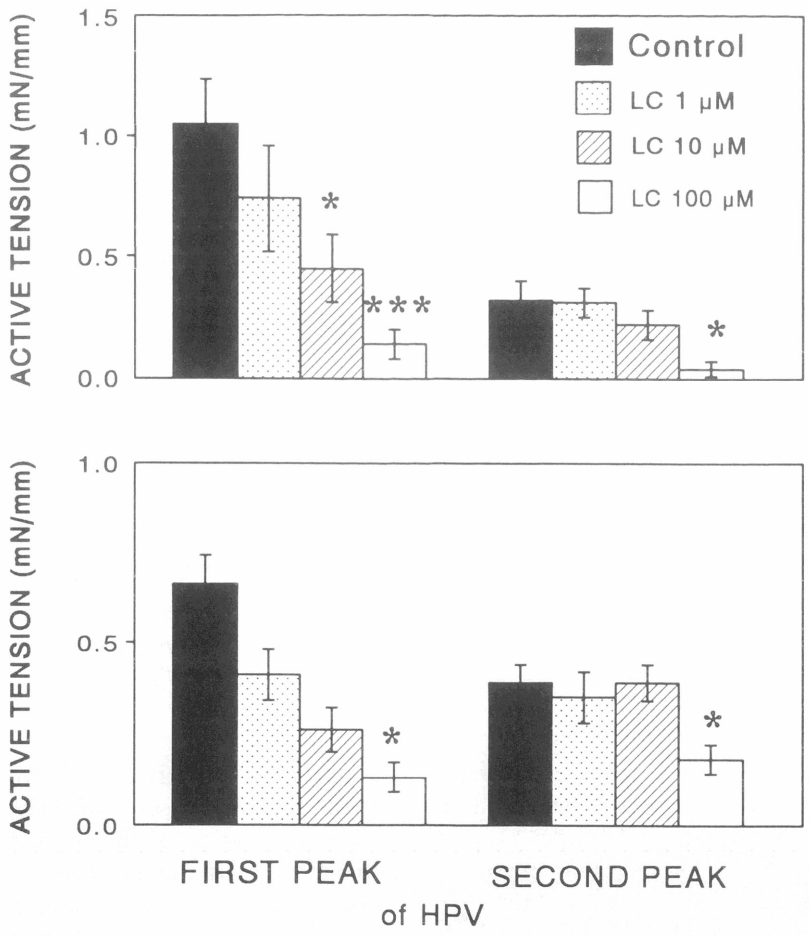


**Fig. 5**
Effect of levcromakalim on hypoxia-induced contractions in PRV (upper panel) and PA (lower panel). Bars show a dose-dependent inhibition of the first peak and the second peak of HPV by levcromakalim (LC). Data are means ±S.E.M., n=8.

**Table 4**
Characteristics of chronic hypoxic (CH) and control (C) pulmonary vessels at equivalent resting tension of 35 mm Hg: Effect of levcromakalim

| | Resistance vessels | | Large arteries | |
|---|---|---|---|---|
| | C | CH | C | CH |
| Internal diameter ($\mu$m) | 246±12 | 217±14 | 1137±60 | 1028±65 |
| Active tension (mN/mm) | 1.5±0.6 | 1.7±0.4 | 1.1±0.2 | 1.8±0.4 |
| Drug concentration (mol/l) | Percentage of contraction | | | |
| $10^{-7}$ | 88.4±2.3 | 74.8±2.8** | 97.8±0.5 | 91.0±2.1** |
| $10^{-6}$ | 77.5±3.9 | 59.1±3.3** | 81.4±4.1 | 82.7±4.1 |
| $10^{-5}$ | 64.7±5.0 | 45.6±4.4* | 59.9±6.7 | 57.1±5.0 |
| $10^{-4}$ | 53.8±6.2 | 33.6±4.0* | 43.3±5.5 | 37.6±6.0 |

*Data are expressed as mean±S.E.M. (n=8) * P<0.05, ** P<0.01, compared to control.*

*Effect of amlodipine on hypoxia-induced contraction*

Table 5 shows that the force of contraction of both phases of HVP was found to be significantly greater in PRV than PA ($p<0.05$). Amlodipine caused a significant dose-dependent inhibition of the first phase of HPV ($P=0.001$), with a significantly greater effect on PRV compared to PA ($P<0.01$). There was a trend towards a dose-independent reduction in the second phase of HPV in PRV, but in PA the second phase tended to increase with dose of amlodipine. The second phase contraction was very variable and none of these changes were significant, with no significant difference between the two vessel sizes.

**Table 5**
The effect of amlodipine on the first and second phases of acute hypoxic pulmonary vasoconstriction

| | PRV | | PA | |
|---|---|---|---|---|
| | First peak of HPV | Second peak | First peak of HPV | Second peak |
| Active tension (mN/mm) (n=37–42) | 2.1±0.3** | 0.6±0.2* | 0.4±0.03 | 0.2±0.03 |
| Drug concentration (mol/l) | Percentage of contraction | | | |
| | n=8 | n=7 | n=10 | n=8 |
| $10^{-7}$ | 78.1±12 | 61.7±8 | 76.4±9 | 70.7±19 |
| $10^{-6}$ | 47.4±8 | 64.2±10 | 87.1±7 | 134.4±31 |
| $10^{-5}$ | 37.6±5 | 54.1±1 | 70.9±8 | 245.2±66 |

*Data in percentage changes from baseline are expressed as means±S.E.M. Values have been adjusted according to the results of the parallel control experiment. * P<0.05, ** P<0.01, compared with PA.*

## Discussion

Our experiments were designed to test the comparability of hypoxic contractions in isolated rat PRV and PA and to investigate the contribution of the endothelium to the hypoxic response. The response of isolated rat vessels to hypoxia has recently been shown to consist of a biphasic contraction (Maxson *et al.* 1989, Rhoades *et al.* 1990). The first phase of the hypoxic response peaks at approximately five minutes and has many of the characteristics, such as response to calcium channel blockade, seen with hypoxic vasoconstriction in

the blood-perfused isolated lung. The second phase of hypoxic vasoconstriction may be endothelium-dependent (Leach *et al.* 1991) and its physiological relevance is less certain. Our results show that the response to hypoxia is a biphasic event both in isolated PRV and PA. We are consistent with Madden *et al.* (1985) and suggest that the large pulmonary arteries (>800 $\mu$m) exhibited a similar but smaller hypoxic contraction. We also suggest that the first phase of HPV was highly reproducible within each vessel size, but the late phase during the second exposure to hypoxia was greater than on the first occasion in both vessel sizes, with great variability between vessels.

Holden and McCall (1984) found that hypoxic contraction in porcine PA was completely endothelium-dependent. In contrast, De Mey and Vanhoutte (1982) found that anoxic contraction in canine pulmonary artery was partially endothelium-dependent, and Burke and Wolin (1987) observed that endothelium-denuded bovine PA rings contract in response to anoxia. In our studies the first phase of the hypoxic response in endothelium-denuded PRV rings is not significantly different compared with the intact endothelium PRV. However, the second phase of the hypoxic response in endothelium-denuded PRV rings was reduced by 68 % compared to that of PRV with intact endothelium. Our results are consistent with the hypothesis that the first phase of the hypoxic response is endothelium-dependent and the second phase is partially endothelium-dependent in isolated rat pulmonary resistance vessels. It is likely that the relative importance of first and second phase responses is different in different species. This may explain some of the conflicting results seen in the literature. If the major component of hypoxic vasoconstriction is the first endothelium-independent phase, then the hypoxic sensor must be located within the vascular smooth muscle cells since there is little else present in the isolated denuded PRV.

Several laboratories have reported the production of HPV in precontracted isolated vessels (Hoshino *et al.* 1988, Teng and Barer 1994). The need for precontraction with agonists such as $PGF_{2\alpha}$ which acts on intracellular calcium stores is surprising if an alteration in membrane permeability to potassium is responsible for HPV. It is possible that up regulation of the contractile apparatus by elevation of intracellular calcium concentration is required before detectable vasoconstriction is seen in isolated vessels of the size studied in these experiments. In the present study we have tried to compare the sensitivity of hypoxic contractions in isolated rat pulmonary vessels with their response to $PGF_{2\alpha}$ and to examine the effects of agents which modify ion flux.

The mechanism of hypoxic contraction in these isolated vessels is unknown, but may involve alterations of potassium flux in pulmonary vascular smooth muscle cells (Yuan *et al.* 1993, Post *et al.* 1992). Recently, several laboratories reported that HPV is associated with potassium channel inhibition. Lopez-Lopez *et al.* (1989), by patch-clamping type I rabbit carotid body cells, found that exposure to hypoxia resulted in a reversible reduction of a potassium current across the cell membrane, flowing down its concentration gradient. Sodium and calcium fluxes were unaffected. This inhibition of potassium channel activity was dependent on the severity of hypoxia between 70 and 120 mm Hg, i.e. it was dose-dependent over the range of $PaO_2$ to which the carotid body might be exposed under physiological conditions. Post *et al.* (1992) examined whether a similar response might be involved in the development of hypoxic pulmonary vasoconstriction. They were able to mimic the effects of hypoxia in isolated rat lungs and pulmonary arterial rings, using three inhibitors of the potassium channel – *Leiurus quinquestriatus* venom, tetraethylammonium (TEA) and 4-aminopyridine. These results were consistent with an earlier study by Ousterhout and Sperelakis (1987) who had found that depolarisation and action potentials resulted when TEA was added to strips of rabbit pulmonary artery.

Cromakalim hyperpolarises the cell membrane in vascular smooth muscle through activation of the ATP-sensitive $K^+$ channels and causes relaxation in a wide range of vascular tissues (Hamilton and Weston 1989, Quast and Cook 1989). The effects of cromakalim are stereospecific, because the (−)-enantiomer (levcromakalim) has a more potent vasodilator action than its racemic form (Buckingham *et al.* 1986, Hof *et al.* 1988). In the present experiment, we found that levcromakalim inhibited the first phase of hypoxic pulmonary vasoconstriction in PA and PRV at all doses tested. It is likely that levcromakalim increases the potassium conductance of the vascular smooth muscle cell membrane. Hyperpolarisation of the membrane, in turn, closes voltage-dependent calcium channels with resultant inhibition of HPV. The second phase of hypoxic contraction was not significantly inhibited by levcromakalim except at high concentration. The other experiment demonstrated that the second phase of the hypoxic response in endothelium-denuded PRV rings was reduced by 68 % compared with intact endothelium PRV. Therefore, we agree with Leach *et al.* (1991) and suggest that the second phase of contraction is more variable and may be partially endothelium-dependent. If a mediator (as yet unknown) is responsible for this phase of hypoxic vasoconstriction, our results would indicate that it acts without involvement of ion channels which would be liable to modulation by membrane hyperpolarisation.

Hypoxic pulmonary vasoconstriction (HPV) has been studied in rat (Rodman *et al.* 1989) and human (Hoshino *et al.* 1988) isolated pulmonary arteries. There is good evidence that activation of voltage-dependent calcium channels is the final step in

the initiation of hypoxic vasoconstriction. Hoshino *et al.* (1988) suggested that hypoxia in the presence of certain other vasoactive agents has a potent contractile effect on the human pulmonary artery and that the response is dependent on $Ca^{2+}$. Our work (Suggett *et al.* 1980) and that of McMurtry (1985) indicated that the calcium channel antagonist verapamil inhibits hypoxic vasoconstriction and that the calcium channel agonist BAY K8644 potentiates hypoxic vasoconstriction in blood-perfused rat lungs. Rodman *et al.* (1989) have demonstrated in isolated rat vessels that hypoxic contractions are highly sensitive to the effects of calcium channel blockade, particularly the resistance vessels. In the present experiment, amlodipine caused a significant dose-dependent inhibition of the first phase of HPV with a significantly greater effect in the resistance vessels than in the large arteries. Despite a trend in the pulmonary resistance vessels, there was no significant inhibition of the second phase of contraction in either size of vessel. The apparent increase in contractile force with increasing amplodipine concentration in large vessels was surprising. The effect was not, however, significant, and is likely to simply reflect the wide variability of the late phase of HPV. We could not rule out the possibility that calcium antagonism would inhibit that phase *in vivo*.

HPV is calcium-dependent, being powerfully inhibited by antagonists of the voltage-dependent calcium channel, such as verapamil (McMurtry *et al.* 1976, Suggett *et al.* 1980), and potentiated by calcium channel agonists, such as BAY K8644 (McMurtry 1985). It is also voltage-dependent, i.e. associated with a decrease in the resting membrane potential of vascular smooth muscle cells, and the development of action potentials (Harder *et al.* 1985, Madden *et al.* 1985). Recently, Farrukh and Michael (1992) suggested that hypoxic pulmonary vasoconstriction directly correlates with extracellular calcium ($[Ca^{2+}]_o$), the absence of which in the perfusate markedly attenuates the hypoxaemia-induced pulmonary vasoconstriction in isolated blood-perfused ferret lungs. Our results demonstrated the significant reduction of the first and second phases of hypoxic pulmonary vasoconstriction both in PRV and PA when $[Ca^{2+}]_o$ is removed. It suggested that the biphasic event is triggered by the influx of $[Ca^{2+}]_o$.

In conclusion, this study has confirmed that hypoxia induces a biphasic contractile response in isolated small and large pulmonary arteries from the rat. The contractile force produced during the first phase of hypoxic contraction was greater than that observed in the second phase in all vessels. A greater force per surface area of vessel was produced during both phases of hypoxia in PRV compared with the equivalent contraction in PA. We suggested that the biphasic event is triggered by the influx of $[Ca^{2+}]_o$. The mechanism of hypoxic vasoconstriction involves direct oxygen sensing by the pulmonary artery. Intact endothelial function is required for maximal response. Our results support the hypothesis that the mechanism of hypoxic vasoconstriction involves direct oxygen sensing by the PRV smooth muscle cells and that the endothelium modulates the hypoxic response, especially in the second phase of HPV. Extracellular calcium is required for HPV and calcium channel antagonist (amlodipine) caused significant dose-dependent inhibition of the first phase of HPV, with a significantly greater effect on PRV compared to PA. In addition, our results showed that the potassium channel opener, levcromakalim, inhibits hypoxic pulmonary vasoconstriction probably by hyperpolarisation of the pulmonary vascular smooth muscle cell membrane and prevention of the opening of voltage-dependent $Ca^{2+}$ channels. The calcium channel antagonist amlodipine inhibited the first phase of HPV, with a significantly greater inhibition demonstrated in PRV compared to PA. The second phase of HPV was not sensitive to amlodipine. These results suggest that levcromakalim and amlodipine may be of value in the treatment of pulmonary hypertension.

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