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EFFECTS OF HYPOXIA UPON CONTRACTIONS EVOKED IN ISOLATED RABBIT PULMONARY ARTERY BY POTASSIUM AND NORADRENALINE

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SUMMARY

1. Comparisons have been made between rabbit thoracic aorta and main pulmonary artery of the effects of hypoxia upon contractions evoked by noradrenaline (NA) and KCl (K^+).

2. Contractions were evoked in cylindrical sections of pulmonary artery and aorta, mounted for isometric recording of tension, by NA and K^+ (at ED_{80}) in normoxia (P_{O_2} 110 mmHg) and hypoxia (P_{O_2} 23 or 7 mmHg). Contractions were also evoked in Ca^{2+}-free conditions with EGTA to prevent influx of extracellular Ca^{2+}. All contractions are expressed as a percentage of normoxic response in the presence of Ca^{2+}.

3. Potassium-evoked contractions of aorta and pulmonary artery were reduced to a similar extent by both levels of hypoxia, to 85 and 92% respectively. As expected K^+-evoked contractions were virtually abolished by Ca^{2+}-free conditions. It is proposed that hypoxia has a small inhibitory effect upon contraction mediated by Ca^{2+} influx via voltage-operated Ca^{2+} channels.

4. In the aorta in the presence of Ca^{2+}, hypoxia reduced NA-evoked contractions to 84% at P_{O_2} 23 mmHg and 34% at P_{O_2} 7 mmHg. In the absence of Ca^{2+}, NA-evoked contractions reached 73% in normoxia, but only 43 and 21% at P_{O_2} 23 and 7 mmHg respectively. These results suggest that hypoxia reduces the component of contraction that is mediated by release of intracellular Ca^{2+} and possibly that mediated by agonist-induced Ca^{2+} influx.

5. In the pulmonary artery also, NA-evoked responses in the absence of Ca^{2+} were reduced from 60% in normoxia, to 49 and 38% at P_{O_2} 23 and 7 mmHg. But, in the presence of Ca^{2+}, hypoxia *potentiated* NA-evoked contractions to 113 and 111% at P_{O_2} 23 and 7 mmHg respectively. It is proposed that in the pulmonary artery, hypoxia reduces the component of contraction mediated by release of intracellular Ca^{2+}, but facilitates that mediated by extracellular Ca^{2+}. Possible mechanisms are discussed.

INTRODUCTION

Oxygen availability is considered to one of the most important local factors influencing vascular contractility (Sparks, 1980). However, the effect of a decrease in oxygen tension is not uniform throughout the vascular system. Thus, in the sys-

temic circulation hypoxia generally induces dilatation which may be functionally important in directing oxygenated blood to hypoxia regions. By contrast, in the pulmonary circulation, hypoxia is associated with vasoconstriction. Such vasoconstriction may constitute part of a negative feedback system which helps improve arterial oxygenation on the lungs by selective redistribution of blood towards the better ventilated alveoli (Dawson, 1984).

Although it is now accepted that hypoxic pulmonary vasoconstriction occurs principally along the arterial tree, probably in the small muscular arteries, there has been debate as to whether the oxygen sensing occurs within the pulmonary vascular muscle itself or in some other supporting tissue (Fishman, 1976; Dawson, 1984). It could be that hypoxia increases the release of vasoconstrictor substance, or decreases the release of a vasodilator substance, either from extravascular tissue, or from some constituent of the blood vessel, which then acts upon the vascular smooth muscle. Investigations using pharmacological blockade have not produced convincing evidence that a single vasoactive substance is responsible for hypoxic vasoconstriction (Barer, 1976; Fishman, 1976; Bergofsky, 1979), although it is possible that some, as yet, undiscovered 'constricting factor' is released during hypoxia (Greenberg & Diecke, 1988). On the other hand, it has been suggested that locally released or circulating vasoconstrictor substances like catecholamines may facilitate endogenous hypoxic constrictor mechanisms by maintaining tone within the vascular muscle (Fishman, 1976; Dawson, 1984).

Many groups have investigated the possibility that hypoxia directly influences pulmonary vascular contractility, principally concentrating upon the possibility that oxygen tension may control various aspects of metabolism (Fishman, 1976; McMurtry, Rounds & Stanbrook, Harris & Heath, 1986); again no mechanism has been conclusively demonstrated. However, it was recently shown that the constrictor effect of hypoxia upon isolated pulmonary arteries of the rat (Hottenstein, Mitzner & Bierkamper, 1984) and the cat (Harder, Madden & Dawson, 1985) was accompanied by membrane depolarization. This raises the possibility that hypoxia causes contraction of pulmonary arteries by facilitating Ca^{2+} entry through voltage-operated channels. In support of this idea it has been shown that hypoxic vasoconstriction in isolated rat lungs can be attenuated by the calcium entry blocker verapamil (McMurtry *et al.* 1982) which is known to be more effective in blocking Ca^{2+} entry through voltage-operated channels than via receptor-operated mechanisms (Cauvin, Loutzenhiser & Van Breeman, 1983).

The present study was designed to examine more fully the mechanisms of hypoxic vasoconstriction using isolated preparations of the rabbit pulmonary artery and, for comparison, the thoracic aorta. The effects of graded hypoxia were examined upon contractions evoked in these arteries by KCl, which is generally considered to induce contraction by causing membrane depolarization, thereby inducing Ca^{2+} influx through voltage-operated channels and by noradrenaline (NA) which may induce contraction by causing Ca^{2+} influx via receptor-operated mechanisms, as well as by causing release of intracellular Ca^{2+}. In addition we examined the effect of hypoxia upon contractions induced by NA in the absence of extracellular Ca^{2+} and in the presence of the calcium entry blocker, verapamil.

Some of these results have already been reported in brief to the Physiological Society (Marriott & Marshall, 1988).

METHODS

The thoracic aorta and left main pulmonary artery were rapidly removed from New Zealand rabbits (2–3 kg body weight) which had been killed by exsanguination under pentobarbitone anaesthesia (45 mg/kg, I.V.). Cylindrical sections of the aorta and pulmonary artery (8 and 5 mm in length respectively) were mounted in organ baths that were in parallel with one another, between parallel tungsten wires (50 μm diameter) under resting tensions of 3 and 2 g respectively which preliminary experiments had shown would put them at the peak of their respective tension–response curves. Tension was monitored via UF1 isometric force transducers (Pioden) and recordings were displayed on a pen recorder (Multitrace 6, Ormed). The arteries were bathed at 37 °C with modified Krebs solution of the following composition (mM): NaCl, 118·40; KCl, 4·75; $CaCl_2$ 2·50; $MgSO_4$, 1·18; KH_2PO_4, 1·19; $NaHCO_3$, 25·00; and glucose, 11·66. EDTA (19 μM) and ascorbic acid (50 μM) were included in the bathing solution in order to reduce the degradation of noradrenaline (NA; Maxwell, Herlihy & Riedel, 1983). During a 1 h equilibration period, the arteries were gassed vigorously with 13% O_2, 5% CO_2 in N_2 (normoxia), the bathing fluid being changed every 20 min. Following equilibration, contractions were evoked by NA (1 μM, aorta, 3 μM, pulmonary artery) or by KCl (30 mM, aorta; 40 mM, pulmonary artery), repeating at 30 min intervals until successive peak responses varied by less than 10% (control responses); each preparation was exposed to only one of the stimulants. The concentrations of NA and KCl used were those which in preliminary experiments produced approximately 80% of their respective maximum responses.

In order to produce hypoxia, the gassing mixture was changed to one containing 2% O_2, 5% CO_2 in N_2, or 5% CO_2 in N_2; each preparation was exposed to only one of the hypoxic mixtures. After 30 min, responses to either NA or KCl were evoked again. Then, the hypoxic gassing mixture was rapidly exchanged for the normoxic mixture without removing the simulant (re-oxygenation). The peak of the tension reached following addition of the stimulant and following re-oxygenation was measured and used for analysis. After removal of the stimulant and equilibration in normoxia for 30 min, further responses could be evoked during hypoxia and following re-oxygenation with less than 10% variation from control responses over the period of the experiment (6 h).

In a few experiments on the pulmonary artery, a different protocol was adopted: the change from one gassing mixture to another was made during the maintained phase of NA-evoked contraction. For some of these experiments, an additional gas mixture to those already mentioned was used (6% O_2, 5% CO_2 in N_2). The gas mixtures were made up in PVC Douglas bags, with the aid of a mass spectrometer.

During each experiment oxygen tensions were continuously measured from the organ baths using Clarke-type polagraphic electrodes (1·35 mm outside diameter) which were placed either in the bulk of the bathing solution, or within 1 mm of the artery surface. These were connected to a two-channel custom-built O_2 meter. The electrodes and meter were calibrated using Krebs solution equilibrated at 37 °C with oxygen mixtures of known composition, atmospheric pressure being taken into consideration. The calibration was cross-checked before and at intervals during each experiment by samples taken anaerobically from the organ baths and measured using Radiometer blood gas analysis equipment (BMS Mk 2, cf. Marriott & Marshall, 1990).

After testing responses to KCl or NA in normoxia in Krebs solution containing Ca^{2+}, the responses of some preparations to KCl or NA were tested in normoxia and in hypoxia in Ca^{2+}-free conditions. For this purpose, the arteries were incubated for 5 min in Krebs solution from which Ca^{2+} had been omitted and 0·5 mM-EGTA added. These Ca^{2+}-free conditions were maintained during the NA- or K^+-evoked response. Measurements were made of the peak contraction. Following re-equilibration in the presence of Ca^{2+} for 30 min, subsequent removal of Ca^{2+} and addition of EGTA allowed further Ca^{2+}-free responses to be elicited which varied by less than 10% from the control Ca^{2+}-free response.

Studies were also made of the effects of verapamil upon NA- and K^+-evoked contractions.

Arteries were pre-incubated with verapamil (10 μM) for 30 min and then without removing verapamil from the baths, responses were evoked by KCl or by NA during normoxia and, during hypoxia followed by re-oxygenation and peak contractions were measured under each of these conditions, as above. Verapamil was added directly to the baths as 0·1 ml of a 1 mM solution in distilled water; in control experiments, addition of 0·1 ml distilled water did not affect control responses.

All results are expressed as the arithmetic mean ± S.E.M. Statistical analyses were made using Student's paired or unpaired t test. Values of $P < 0.05$ were considered significant.

TABLE 1. Levels of P_{O_2} measured in organ baths at equilibration with gas mixtures of known composition

Gas mixture (% O_2)	P_{O_2} (mmHg)	n
13	107 ± 2	736
7	54 ± 3	96
2	23 ± 3	224
0	7 ± 1	308

Each gas mixture contained 5% CO_2, the balance was N_2. n indicates number of instantaneous measurements made with O_2 electrode and cross-checked with Radiometer equipment (see text).

RESULTS

When the Krebs solution in the organ baths was gassed with the 13, 7, 2 and 0% O_2 mixtures, and equilibrium P_{O_2} in the baths was reached in less than 5 min; the values measured are shown in Table 1.

The effects of changes P_{O_2} upon contractility

Potassium-evoked contractions

Exposure of the aorta to hypoxia (P_{O_2} 23 and 7 mmHg) reduced K^+-evoked contractions; the effects were not graded with the level of hypoxia (Fig. 2). Thus, at a P_{O_2} of 23 and 7 mmHg, K^+-evoked contractions of the aorta reached 85 ± 3 and 86 ± 4% respectively of the normoxic control response, the reduction reaching significance at P_{O_2} 7 mmHg. Upon re-oxygenation these contractions recovered to 91 ± 3 and 103 ± 3% which were not significantly different from the original normoxic control responses (see Fig. 2). In the pulmonary artery, the mean effects of hypoxia on K^+-evoked contractions was somewhat less than for the aorta; they were reduced to 93 ± 2 and 94 ± 5% at P_{O_2} 23 an 7 mmHg respectively of normoxic responses, the reduction reaching significance at P_{O_2} 23 mmHg. Upon re-oxygenation these contractions recovered to 103 ± 3 and 107 ± 4% respectively of the normoxic response (Fig. 2), values that were not significantly different for the original normoxic response.

Noradrenaline-evoked contractions

In the aorta, reduction in P_{O_2} to 23 or 7 mmHg caused graded reduction of NA-evoked contractions; they reached 84 ± 4 and 34 ± 7% of the normoxic control responses respectively (Figs 1*A* and 2). They was no obvious difference between the shapes of the NA-evoked contractions of the aorta in normoxia and hypoxia

(Fig. 1*A*). As can be seen in Fig. 2, at P_{O_2} 7 mmHg the NA-evoked contractions of the aorta were reduced significantly more than the K^+-evoked contractions. Re-oxygenated after exposure to P_{O_2} 23 mmHg restored the NA-evoked contractions to $103 \pm 3\%$ of the normoxic control, but re-oxygenation from P_{O_2} 7 mmHg produced recovery to only $75 \pm 6\%$ of the normoxic control response, a level which was significantly different from that control response.

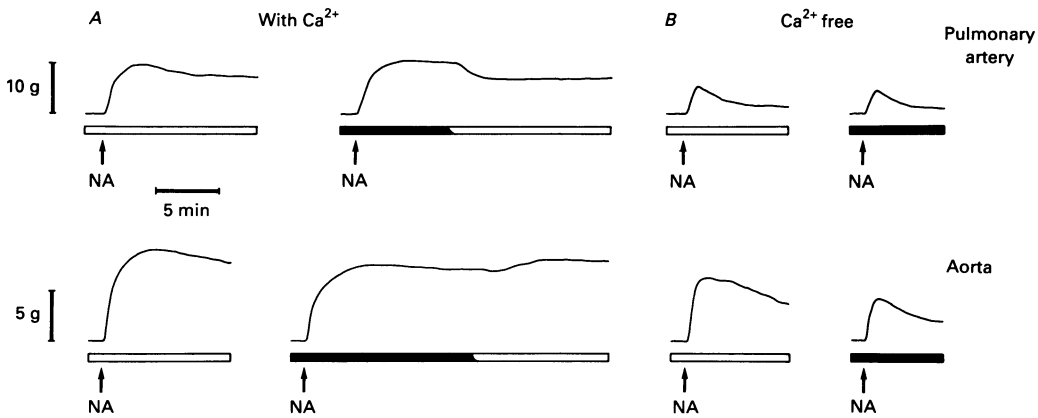

Fig. 1. Traces of responses evoked by noradrenaline (NA) in a pulmonary artery (above), and an aorta (below) in normoxia and in hypoxia (*A*) and Ca^{2+}-free conditions in normoxia and hypoxia (*B*). Calibration bars: vertical bars indicate magnitude of contraction (g); horizontal, time (min). Open bars beneath traces indicate normoxia (P_{O_2} 107 mmHg).

By contrast, in the pulmonary artery, hypoxia potentiated NA-evoked contractions; they reached 113 ± 6 and $111 \pm 9\%$ of the normoxic control response during exposure to P_{O_2} 23 and 7 mmHg respectively, the increase being significant at P_{O_2} 23 mmHg (Figs 1*A* and 2). The effects of hypoxia upon K^+- and NA-evoked contractions of the pulmonary artery were significantly different at P_{O_2} 23 mmHg (Fig. 2). As can be seen from Fig. 1*A*, while in normoxia NA-evoked contractions of the pulmonary artery tended to decline after reaching an initial peak, as did those of the aorta both in normoxia and hypoxia. By contrast, the NA-evoked contractions of the pulmonary artery in hypoxia were well maintained for up to 5 min. Re-oxygenation from P_{O_2} 23 and 7 mmHg produced relaxation of the pulmonary arteries; to 81 ± 7 and $57 \pm 9\%$ of normoxic control responses respectively and in both cases these levels of contraction were significantly smaller than the original contraction evoked by NA in normoxia (Figs 2 and 3*C*).

We also found that if pulmonary arteries were contracted by NA under normoxic conditions (P_{O_2} 107 mmHg) and then P_{O_2} was reduced to 7 mmHg, there was augmentation of the maintained contraction (see Fig. 3*A*). A similar effect was observed when contraction was evoked in pulmonary arteries which were equilibrated at P_{O_2} 54 mmHg, for a change to P_{O_2} 7 mmHg significantly augmented the maintained NA-evoked contraction (Fig. 3*B*) from 82 ± 11 to $148 \pm 20\%$ ($n = 4$, $P < 0{\cdot}05$) of the normoxic control. This effect was apparently opposite to the observation made above that when pulmonary arteries were contracted by NA at P_{O_2} 7 mmHg and then P_{O_2} was increased to 107 mmHg the maintained contraction was substantially reduced (Fig. 3*C*).

Effects of Ca^{2+}-free conditions

As above, all contractions are considered as percentages of the control responses, i.e. that evoked in normoxia in the presence of Ca^{2+}. In normoxia and at both levels of P_{O_2} the contractions evoked by K^+ in Ca^{2+}-free Krebs solution in both aorta and

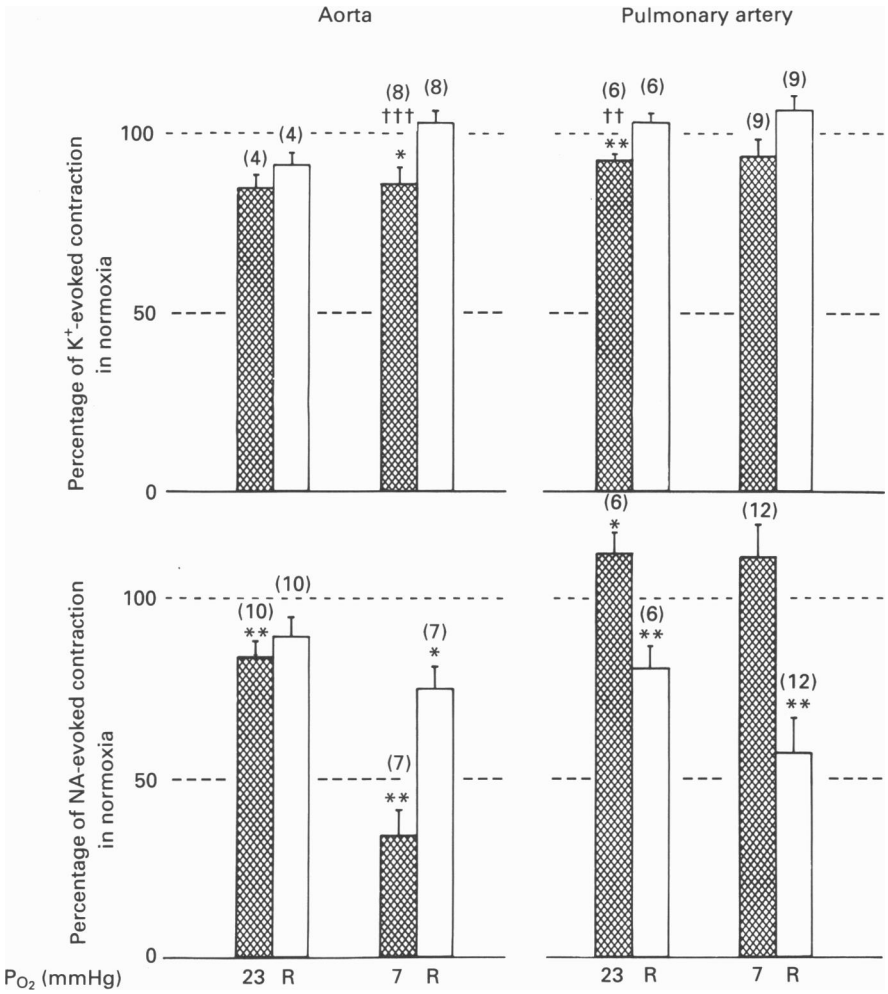

Fig. 2. Effects of two levels of hypoxia and of re-oxygenation on responses evoked by K^+ (above) and noradrenaline (NA) (below) in aorta (left-hand side) and pulmonary artery (right-hand side). Vertical axis indicates magnitude of contraction at percentage contraction evoked in normoxia (P_{O_2} 107 mmHg). Horizontal dashed lines represent 50 and 100% of control response. Cross-hatched columns, responses evoked in hypoxia (P_{O_2} values indicated below in mmHg). Open columns, responses upon re-oxygenation. *, ** indicate significant differences ($P < 0{\cdot}05$, 0·1 respectively) from the normoxic control. ††, ††† indicate significant difference (0·01, 0·001 respectively) between K^+- and NA-evoked contractions. Numbers in parentheses, number of measurements.

pulmonary artery were < 5% of their respective control responses ($P < 0{\cdot}001$, for each artery, at each P_{O_2}; $n > 5$ in every case).

By contrast, NA still evoked substantial contraction of both the aorta and

pulmonary artery under Ca^{2+}-free conditions. These were transient contractions compared with those evoked in Krebs solution containing Ca^{2+} (Fig. 1*B*). In normoxia their peaks reached 73±2 and 60±3% of the control responses in aorta and pulmonary artery respectively (see Fig. 4*A c* and *d*). In the aorta, exposure to

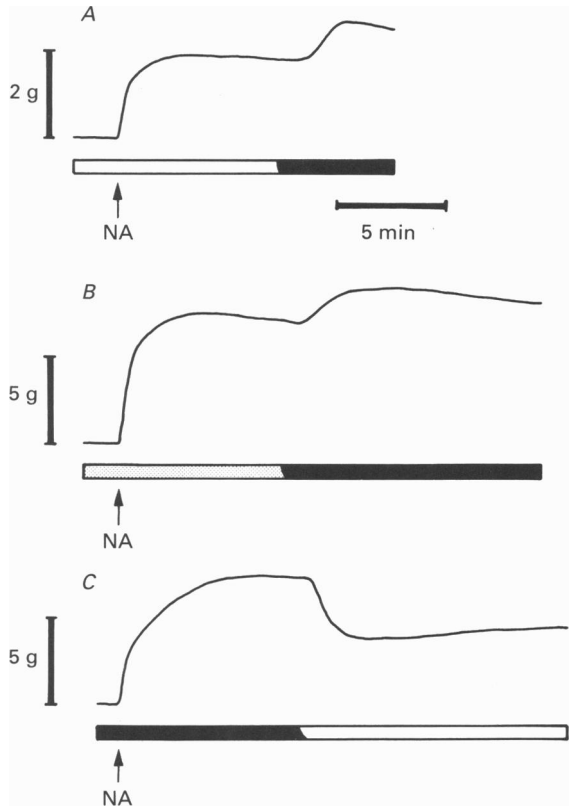

Fig. 3. Examples of effects produce by changing P_{O_2} during noradrenaline (NA)-evoked contraction in three different pulmonary arteries. *A*, contraction evoked by NA in P_{O_2} 107 mmHg (open bar), P_{O_2} changed to 7 mmHg at filled bar. *B*, contraction evoked by NA in P_{O_2} 54 mmHg (stippled bar), P_{O_2} changed to 7 mmHg at filled bar. *C*, contraction evoked by NA in P_{O_2} 7 mmHg (filled bar), P_{O_2} changed to 107 mmHg at open bar. Calibration bars: vertical, magnitude of contraction (g); horizontal, time (min).

P_{O_2} 23 and 7 mmHg caused significant and graded reduction of the Ca^{2+}-free responses; they reached 43±5 and 21±5% respectively of the control response (Figs 1*B* and 4*A c*). Similarly, exposure to P_{O_2} 23 and 7 mmHg reduced the Ca^{2+}-free responses of the pulmonary artery responses, to 49±4 and 38±5% respectively of the control response (Figs 1*B* and 4*A d*), though the reduction only reached significance at P_{O_2} 7 mmHg.

The effects of verapamil

Potassium-evoked contractions

Again, contractions are considered as percentages of the control response, i.e. that evoked in normoxia in the absence of verapamil. Verapamil (10 μM) greatly reduced

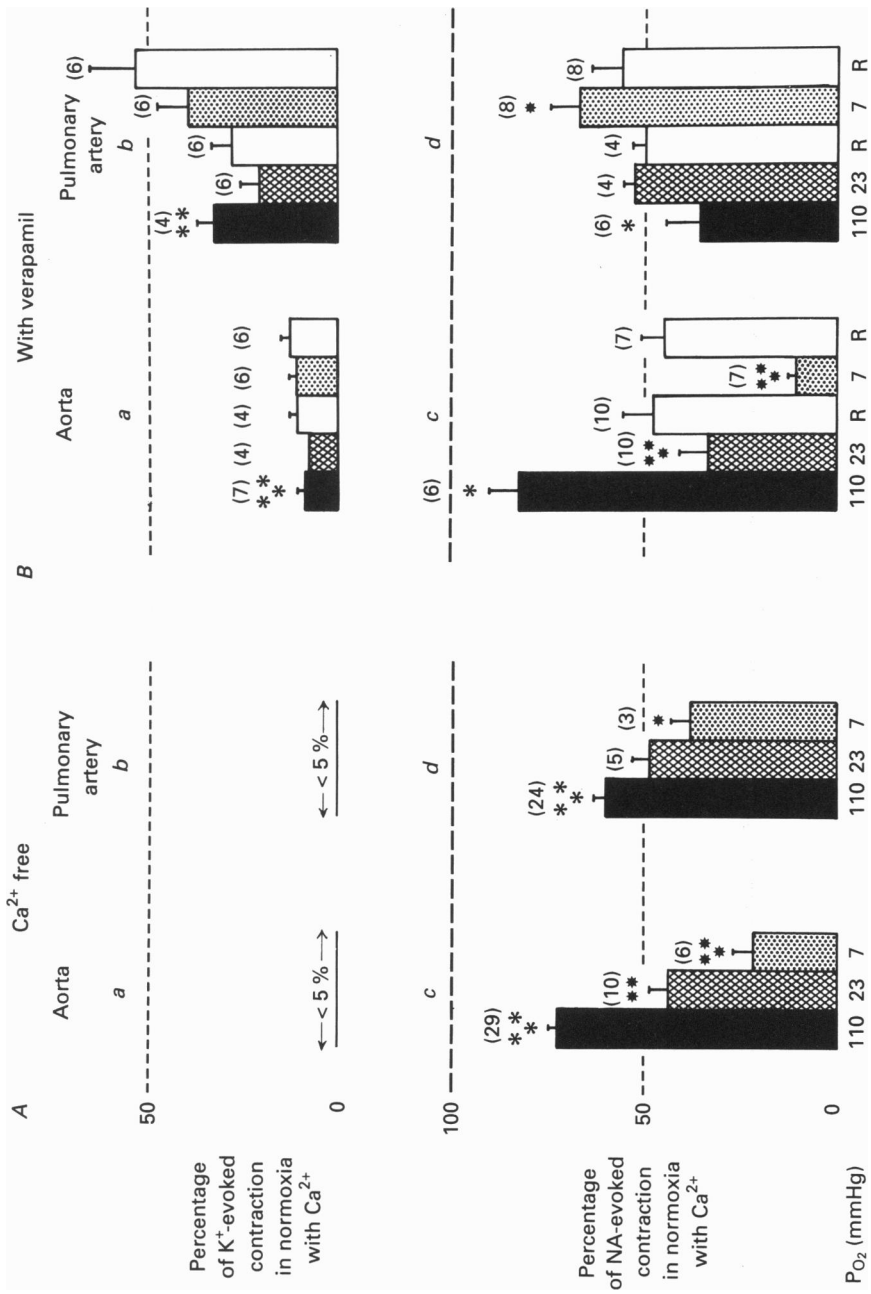

Fig. 4. For legend see facing page.

K^+-evoked contractions of the aorta in normoxia and at both levels of hypoxia, to $< 10\%$ of the control response (Fig. 4*Ba*).

By contrast, in the pulmonary artery verapamil allowed substantial contractions to be evoked by K^+ in normoxia and at both levels of hypoxia (Fig. 4*Bb*). In normoxia, K^+-evoked contractions reached $32{\cdot}4 \pm 4{\cdot}3\%$ ($n = 4$) of the control response and at P_{O_2} 23 and 7 mmHg they reached 21 ± 5 and $39 \pm 8\%$ respectively of the control response. There was no significant difference between the contractions evoked by K^+ in the pulmonary artery in the presence of verapamil in normoxia and at either level of hypoxia. Re-oxygenation from both levels of hypoxia appeared to allow some enhancement of the contractions, to 27 ± 5 and $52 \pm 12\%$ respectively of the control response (Fig. 4*Bb*).

Noradrenaline-evoked contractions

In normoxia, verapamil significantly reduced NA-evoked contractions of the aorta to $83 \pm 8\%$ of the control response (Fig. 4*Bc*). During hypoxia, in the presence of verapamil, the NA-evoked responses were further reduced in a graded fashion to 33 ± 7 and $11 \pm 2\%$ of the control responses at P_{O_2} 23 and 7 mmHg respectively and upon re-oxygenation these responses recovered significantly to 48 ± 8 and $45 \pm 6\%$ respectively (Fig. 4*Bc*, $P < 0{\cdot}05$, contraction in hypoxia *vs.* contraction upon re-oxygenation).

Verapamil also caused substantial reduction of the NA-evoked responses of the pulmonary artery in normoxia to $36 \pm 10\%$ of the control response (Fig. 4*Bd*). But, in hypoxia in the presence of verapamil, NA-evoked contractions of the pulmonary artery were larger than those evoked in normoxia in the presence of verapamil; they reached 52 ± 2 and $67 \pm 7\%$ of normoxic controls at P_{O_2} 23 and 7 mmHg respectively, the difference between the normoxic and hypoxic response in the presence of verapamil reaching significance at P_{O_2} 7 mmHg (Fig. 4*Bd*). Upon re-oxygenation these contractions reached 50 ± 3 and $56 \pm 8\%$ respectively of the control normoxic responses, values that were not significantly different from the hypoxic responses (Fig. 4*Bd*).

DISCUSSION

In the present experiments on the aorta, a reduction in P_{O_2} from 107 to 23 or 7 mmHg, produced a similar, rather small reduction in the magnitude of K^+-evoked

Fig. 4. Contractions evoked in normoxia and at two levels of hypoxia by K^+(above) and NA (below) in Ca^{2+}-free conditions (*A*), and in presence of verapamil (*B*). In *A* and *B* responses of aorta are shown on left (*a* and *c* in each case), those of pulmonary artery on right (*b* and *d* in each case). Vertical axis indicates magnitude of contraction expressed as a percentage of the control response, i.e. the contraction evoked in normoxia in presence of Ca^{2+}. Horizontal dashed lines represent 50 and 100% of control response. Filled columns, responses evoked in normoxia (P_{O_2} 107 mmHg); cross-hatched columns, responses evoked in P_{O_2} 7 mmHg; open columns, responses upon re-oxygenation. * indicates significant differences between response evoked in normoxia in presence of Ca^{2+} and those evoked in normoxia in absence of Ca^{2+}, or in presence of verapamil. ✱ indicates significant differences between responses evoked in normoxia and hypoxia in Ca^{2+}-free conditions, or in presence of verapamil (1, 2 and 3 symbols indicate $P < 0{\cdot}05$, 0·01, 0·001 respectively). Numbers in parentheses, number of measurements.

contractions, but produced more substantial and graded reduction of NA-evoked contractions. This accords with our observations on a range of systemic arteries of the rabbit, viz. femoral, saphenous and renal arteries (Marriott & Marshall, 1990). By contrast, in the pulmonary artery although K^+-evoked contractions were slightly reduced by both levels of hypoxia, contractions evoked by NA were larger at P_{O_2} 23 and 7 mmHg than at P_{O_2} 107 mmHg. Moreover, a change to P_{O_2} 7 mmHg from either 100 or from 54 mmHg, augmented an existing NA-evoked contraction. These findings seem relevant to behaviour that might occur in the pulmonary artery *in vivo* upon reduction of P_{O_2}, for a while the wall of the pulmonary artery is supplied with oxygenated blood through a well-developed vasa vasorum (Harris & Heath, 1986), the P_{O_2} of the mixed venous blood that perfused the lumen of the pulmonary artery would normally be 45–50 mmHg.

Whilst pulmonary arterial vessels constrict in response to hypoxia *in situ*, there have been few demonstrations of this in isolated pulmonary arteries (see Fishman, 1976). Lloyd (1968) who used small pulmonary arteries that were not under the influence of an agonist (non-stimulated) demonstrated hypoxia-induced contractions, but only after 60–80 min incubation in fluorochemical substrate at P_{O_2} 650 mmHg and in the presence of a high concentration of procaine; he concluded that it was necessary for some parenchyma to be attached to the vessel. Later Lloyd (1970) showed that hypoxia could induce constriction in non-stimulated strips of main pulmonary artery than were devoid of parenchyma. But these vessels were incubated in humid gas for 2–3 h and therefore were not supplied with substrate. More recently, Hottenstein *et al.* (1984) and Harder *et al.* (1985) found that hypoxia contracted non-stimulated pulmonary arteries of the rat and cat respectively, that were in physiological salt solution. But, Harder *et al.* (1985) emphasized that the main pulmonary arteries of the cat, comparable anatomically to those we used, were not consistently contracted by hypoxia and the responses that did occur were small.

It could be that the constrictor effect of hypoxia is more readily demonstrated in pulmonary arteries that are partly contracted by an agonist. This would accord with evidence that a basal tone is required for the demonstration of hypoxic vasoconstriction in whole-lung preparations (see Fishman, 1976). Certainly, Detar & Gellai (1971) reported that hypoxia consistently enhanced contractions that were evoked in rabbit pulmonary artery by adrenaline, acetylcholine and KCl. However, the extent to which this occurred depended on the control P_{O_2} and the period of equilibration at that P_{O_2}: pulmonary arteries had to be equilibrated at P_{O_2} 100 mmHg for as long as 22 h, or at P_{O_2} 40 mmHg for only 2–6 h before they would show further contraction on exposure to P_{O_2} 0 mmHg and relaxation on return to P_{O_2} 100 mmHg. Moreover, Detar & Gellai (1971) also found that rabbit aortae equilibrated at P_{O_2} 40, 20 or 0 mmHg for 6–8 h showed similar behaviour to the pulmonary arteries. Since these effects took so long to develop and occurred in aorta as well as pulmonary artery, it may be that Detar & Gellai (1971) were observing a different phenomenon from us.

There is already substantial evidence that hypoxia-induced *relaxation* of systemic arteries cannot be explained simply by limitation of the O_2 supply to support aerobic metabolism and thereby contraction. Our finding that NA-evoked contraction of aorta and other systemic arteries were reduced far more than K^+-evoked contrac-

tions supports that view (see Marriott & Marshall, 1990 for further discussion). Correspondingly, the directionally different effects of hypoxia upon K^+- and NA-evoked contractions of the pulmonary artery could not be attributed solely to effects in intermediary metabolism, even if it is assumed that pulmonary vascular muscle is better adapted for anaerobic than aerobic metabolism. This is consistent with the proposal made by Detar & Bohr (1972) who speculated that hypoxia-induced contraction may be due in part to an elevation of intracellular Ca^{2+}. Below, we have considered the possible effects of hypoxia upon Ca^{2+} handling in the pulmonary artery, making use of comparisons between it and the aorta.

Potassium-evoked contractions

Potassium chloride is known to contract vascular smooth muscle by depolarizing the cell membrane and allowing Ca^{2+} influx via voltage-operated channels (VOCs) (Weiss, 1985). Our finding that Ca^{2+}-free conditions virtually abolished K^+-evoked contractions in both aorta and pulmonary artery is consistent with that view. The fact that K^+-evoked contractions of both arteries were slightly reduced by hypoxia could therefore be explained if hypoxia inhibited Ca^{2+} influx via VOCs, albeit to a small extent. Indeed, hypoxia inhibited K^+-induced ^{45}Ca uptake of the rabbit aorta (Ebeigbe, Jennett & Pickard, 1980). Re-oxygenation in the presence of KCl allowed both the aorta and pulmonary artery to regain a degree of contraction comparable to that evoked by KCl in normoxia, suggesting that inhibition of the K^+-evoked contraction was easily reversed (cf. Marriott & Marshall, 1990).

Noradrenaline-evoked contractions

It was not surprising that NA evoked contractions in both aorta and pulmonary artery in Ca^{2+}-free Krebs solution, for NA can cause contraction of vascular smooth muscle by releasing Ca^{2+} from intracellular stores, as well as by causing Ca^{2+} influx via receptor-operated mechanisms and, if there is sufficient depolarization of the muscle membrane, via VOCs (Weiss, 1985). The fact that in both vessels the contraction evoked by NA in Ca^{2+}-free solution was reduced in a graded fashion by exposure to graded hypoxia accords with our previous findings on systemic arteries (Marriott & Marshall, 1990).

If all contractions are considered as percentages of the contraction evoked by NA in normoxia in the presence of Ca^{2+}, then for the aorta at P_{O_2} 23 mmHg the apparent reduction of the component mediated intracellular release of Ca^{2+} (Fig. 4*A c*) would easily account for the reduction of the response evoked in the presence of Ca^{2+} (cf. Fig. 2). However, at P_{O_2} 7 mmHg, the reduction of the contraction evoked in the presence of Ca^{2+} was greater than that evoked in the absence of Ca^{2+} (66 *vs.* 52%, cf. Fig. 2 and 4*A d*). Although other interpretations are feasible, this discrepancy raises the possibility that in the aorta hypoxia also reduces the component of contraction that is mediated by NA-induced influx of extracellular Ca^{2+} (cf. Marriott & Marshall, 1990). Accordingly Ebeigbe (1982) showed that hypoxia (P_{O_2}, 15 mmHg) inhibited NA-induced ^{45}Ca uptake by rabbit aorta.

The general finding for rabbit systemic arteries has been that a concentration of NA sufficient to evoke 80% maximal contraction, as we used, does not depolarize vascular smooth muscle (Holman & Suprenant, 1980). Thus, in the aorta, hypoxia

may reduce the influx of extracellular Ca^{2+} through receptor-operated mechanisms. Indeed, as contractions evoked by NA both in the presence and absence of Ca^{2+} were markedly reduced by hypoxia, whereas K^+-evoked contractions were only slightly reduced, it seems that the receptor-operated processes that increase cytosolic Ca^{2+} levels are particularly vulnerable to the actions of hypoxia (cf. Marriott & Marshall, 1990).

Since in the pulmonary artery contractions evoked by NA in the presence of Ca^{2+} were potentiated by hypoxia and since the component of the NA-evoked contraction that was mediated by release of intracellular Ca^{2+} was reduced by hypoxia (Fig. 4*A*), it seems that in this artery, hypoxia must have substantially *potentiated* the component of the NA-evoked contraction that was mediated by extracellular Ca^{2+}. A concentration of 3 μM-NA, which evokes 80% of maximum contraction, would be expected to depolarize the smooth muscle of the rabbit pulmonary artery from about -60 mV, by approximately 15 mV (Haeusler, 1983). Such depolarization is normally the most that can be produced in pulmonary artery by NA; it is achieved by a concentration of only 1 μM-NA and is insufficient to open VOCs (Haeusler, 1983). Thus, in our study, NA-induced influx of Ca^{2+} in normoxia probably occurred via receptor-operated mechanisms.

A hypothesis that would be compatible with these findings is that if hypoxia itself tends to depolarize pulmonary arteries, as reported by Harder *et al.* (1985) and Hottenstein *et al.* (1984), additional NA-induced depolarization may then be sufficient to open VOCs allowing additional influx of Ca^{2+} to potentiate the contraction. In other words, hypoxia may facilitate Ca^{2+} influx via VOCs by indirect, rather than direct means, Our finding that hypoxia did not facilitate K^+-evoked contractions of the pulmonary artery would not be inconsistent with this hypothesis since any tendency for hypoxia-induced depolarization to facilitate the depolarization induced by K^+ might be opposed by a more direct inhibition Ca^{2+} influx via VOCs (see above). This seems particularly likely as the concentration of K^+ we used to evoke contraction would be near the plateau of the K^+ concentration–response curve (Haeusler, 1983).

When still in the presence of NA, re-oxygenation increased the contraction of the aorta towards but not to equal that evoked by NA in normoxia. We observed similar behaviour in rabbit saphenous artery, although the renal and femoral artery readily regained the normoxic level of contraction upon re-oxygenation from both P_{O_2} 23 and 6 mmHg (Marriott & Marshall, 1990). On the other hand, re-oxygenation of the pulmonary artery induced relaxation to well below the control contraction evoked by NA in normoxia. The mechanisms underlying these effects are unclear. However, as the contraction evoked anew by NA in normoxia, was in all vessels, comparable in magnitude with the original normoxic control response (cf. Marriott & Marshall, 1990), hypoxia apparently had no long-term effect on NA-evoked contraction.

Effects of verapamil

The influences of verapamil may be explained in terms of the proposals made above. In normoxia verapamil virtually blocked K^+-evoked contractions of the aorta, but reduced those evoked by NA to a similar extent as did Ca^{2+}-free conditions. This accords with the evidence that verapamil blocks Ca^{2+} entry and is

particularly effective in blocking Ca^{2+} entry via VOCs (Cauvin *et al.* 1983). That contractions evoked in the aorta by NA in the presence of verapamil were inhibited in a graded fashion by graded hypoxia accords with our proposal that hypoxia inhibits the components of contraction mediated by receptor-operated Ca^{2+} influx and release of intracellular Ca^{2+}.

The VOCs of the pulmonary artery were apparently not as sensitive to verapamil as those of the aorta or other systemic arteries (Marriott & Marshall, 1990) for 10 μM-verapamil failed to block a substantial portion of the K^+-evoked contraction in the pulmonary artery both in normoxia and in hypoxia. Thus, verapamil did not enable us to test the hypothesis that the potentiation of contraction induced by hypoxia was mediated via VOCs, whether directly or indirectly (cf. Hottenstein *et al.* 1984). However, given an incomplete block of VOCs, the fact that hypoxia potentiated contractions evoked in the pulmonary artery by NA in the presence of verapamil could be explained if hypoxia itself induced depolarization and allowed NA to induce Ca^{2+} influx via VOCs.

It remains to be seen whether the effects we have described in main pulmonary artery can be observed in the smaller arterial vessels that determine pulmonary vascular resistance. However, our findings may be of relevance to the control of pulmonary vascular compliance. The large pulmonary arteries are under the tonic constrictor influence of sympathetic noradrenergic fibres (Fishman, 1976). Moreover, an increase in circulating levels of catecholamines, or a reflex increase in sympathetic activity, evoked for example by hypoxic stimulation of peripheral chemoreceptors, or by other noxious stimuli as part of an alerting/defence response, can increase the tone of the large pulmonary arteries, and so significantly decrease the compliance of the pulmonary circulation (Szidon & Fishman, 1969, 1971). A tonic constrictor influence would be expected to produce a background against which a fall in the P_{O_2} of mixed venous blood, or in the arterial supply to the vasa vasorum, would be decrease pulmonary vascular compliance. On the other hand, abnormally low levels of P_{O_2} within the lumen, or wall of pulmonary arteries, might accentuate any decrease in compliance produced by circulation catecholamines or by sympathetic activity.

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