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# Effects of hypoxia on the pharmacological responsiveness of isolated coronary artery rings from the sheep

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**1** The effects of low oxygen tension on tone and on the responsiveness to contractile and relaxant agents were examined on circumflex coronary artery rings isolated from sheep.

**2** When artery rings (2–2.5 mm o.d.) were set at their optimal resting tension, introduction of hypoxia (0% $O_2$) caused a sustained contraction which was reversible on washing with oxygenated Krebs solution. In precontracted (40 mM KCl) arteries, hypoxia caused a similar response except that it was preceded by a transient relaxation.

**3** The hypoxia-induced contraction was potentiated by the combination of phentolamine (1 $\mu$M) and propranolol (1 $\mu$M), markedly reduced by verapamil (10 $\mu$M) and either abolished or reduced by indomethacin (1 $\mu$M). Indomethacin itself caused a contraction.

**4** Under hypoxic conditions, the contractile effects of U46619 (a stable thromboxane analogue) and 5-hydroxytryptamine (5-HT) and the vasodilator effects of noradrenaline, iloprost (a prostacyclin mimetic) and adenosine were markedly potentiated. In contrast, vasoconstriction to potassium or acetylcholine was depressed.

**5** Changing the gases from 95% $O_2$ to 12% $O_2$ had no significant effect on the contractile effects of U46619. However, the maximum contractile effect of U46619 was significantly enhanced by changing the gases from 12% $O_2$ to 0% $O_2$.

**6** Rings from a smaller branch (0.6–1.3 mm o.d.) of the circumflex coronary artery of the sheep, in the presence of hypoxia, exhibited qualitatively similar changes in the responsiveness to U46619, 5-HT and adenosine to those observed in the large artery. However, the effect of potassium was potentiated rather than depressed.

**7** It is concluded that hypoxia-induced contraction may involve a modified release of cyclooxygenase products and be partly dependent upon the availability of extracellular calcium.

**8** The change in the responsiveness of coronary arteries, under hypoxia, to both constrictor and dilator mediators may have clinical relevance to myocardial ischaemia and angina pectoris.

## Introduction

In canine (Borda *et al.*, 1980; Rubanyi & Vanhoutte, 1985) and porcine (Rubanyi & Paul, 1985) isolated coronary arteries, hypoxia, one of the consequences of myocardial ischaemia, has been found to cause contraction. Furthermore, hypoxia has been shown to augment the contractile responses to 5-hydroxytryptamine (5-HT) and noradrenaline in isolated canine coronary arteries (Van Neuten & Vanhoutte, 1980). In addition to 5-HT (from clotting platelets) and noradrenaline (from sympathetic nerve endings), many other chemical mediators that modify coronary arterial tone are released during myocardial ischaemia. These include thromboxane $A_2$, prostacyclin, adenosine, potassium and acetylcholine (Yasue *et al.*, 1974; Folts *et al.*, 1976; Berne, 1980; Coker *et al.*, 1981; Kleber, 1984). However, it is not known whether the effects of these mediators on the coronary artery are modified under conditions of hypoxia.

The present experiments were designed firstly, to characterize the effect of hypoxia on the tone of isolated coronary arteries from the sheep and secondly to examine the responsiveness of these arteries, under hypoxia, to a range of substances thought to be released during myocardial ischaemia. The substances investigated were $K^+$, 5-HT, U46619 (the stable analogue of thromboxane $A_2$), noradrenaline, acetylcholine, iloprost (a prostacyclin mimetic)

and adenosine. A preliminary account of these findings has been presented to the British Pharmacological Society (Kwan *et al.*, 1988).

## Methods

Experiments were performed on rings of left circumflex coronary arteries of two sizes: o.d. = 2–2.5 mm (main circumflex trunk after its first branch) and o.d. = 0.6–1.3 mm (proximal portion of second generation branch off main circumflex). The coronary arteries were dissected free from hearts of freshly slaughtered sheep and cleared of fat and adhering connective tissue before cutting into rings 4–5 mm long. Care was taken to avoid stretching and damage to the luminal surface. Lack of damage was confirmed by histological examination in a representative sample of artery rings. Rings were suspended in a water-jacketed muscle chamber (10 ml) filled with Krebs-Henseleit solution (37°C) of the following composition (mM): NaCl 119, $NaHCO_3$ 25, glucose 11, KCl 4.6, $MgCl_2$ 1.2, $KH_2PO_4$ 1.2, $CaCl_2$ 2.5. The solution was aerated with gas mixtures containing 95% $O_2$: 5% $CO_2$ (oxygenated), 95% $N_2$: 5% $CO_2$ (hypoxic), or 12% $O_2$: 5% $CO_2$ in $N_2$ (normoxic).

The rings were suspended on a pair of stainless-steel hooks, one of which was fixed to an L-shaped rod inside the chamber and the other to a Grass isometric transducer. Arterial rings were equilibrated in Krebs-Henseleit solution gassed with 95% $O_2$: 5% $CO_2$ for an hour at their optimum resting tension of 1.5 g (o.d. = 2–2.5 mm) or 1.4 g (o.d. = 0.6–1.3 mm). The optimum resting tension of each size of artery was determined in eight preparations by comparing the tension developed by 40 mM KCl under different resting tensions. The isometric tension was calculated as force developed per cross sectional area. The cross sectional area (A) of the artery was calculated by using the equation: A = blotted weight of the artery/(h × $\beta$) where h = the distance (cm) between the two stainless steel hooks with the artery ring under optimum resting tension, and $\beta$ = the density of the artery ring which was taken as 1.05 g $cm^{-3}$. The tissues were subjected to repeated contraction cycles with 40 mM KCl until two consecutive identical responses were observed before starting the experimental protocol.

Initially the oxygen tension of the bathing medium was measured using a blood gas analyser (Radiometer, Copenhagen). This necessitated removal by syringe of an aliquot of the bathing medium and subsequent injection into the gas analyser. In the course of these experiments, however, it was determined that this procedure resulted in contamination of the sample with atmospheric oxygen. For example, the $Po_2$ of the bathing medium equilibrated with 0% $O_2$ measured using the gas analyser and an oxygen electrode inserted into the oxygen bath was 44 ± 3 and 8 ± 2 mmHg, respectively ($n$ = 3). Therefore all $Po_2$ values quoted are those measured with an oxygen electrode (Strathkelvin Instruments) immersed in the organ bath. This electrode was calibrated to zero every two weeks using sodium sulphite 100 mM in sodium tetraborate 10 mM. The pH and $Pco_2$ of the solution was measured using the gas analyser.

### *Experimental protocols*

*Effect of different oxygen tensions on the tone of the coronary artery:* Hypoxia was introduced by changing the oxygenated Krebs-Henseleit solution (95% $O_2$: 5% $CO_2$) with one Krebs-Henseleit solution 95% $N_2$: 5% $CO_2$. The organ bath was then bubbled continuously with 95% $N_2$-5% $CO_2$ for 25 min and $Po_2$ values (mmHg) of 21 ± 3, 16 ± 2, 10 ± 2, 9 ± 2, 8 ± 2, were obtained at 30 s, 2, 5, 15, and 25 min respectively after the addition of hypoxic Krebs-Henseleit solution. Oxygenated conditions were re-established by washing with Krebs-Henseleit solution aerated with 95% $O_2$-5% $CO_2$. In some experiments, the effect of changing the gas from 95% $O_2$: 5% $CO_2$ to 12% $O_2$: 5% $CO_2$: 83% $N_2$ and from 12% $O_2$: 5% $CO_2$: 83% $N_2$ to 95% $N_2$: 5% $CO_2$ were examined. The $Po_2$ values in the organ bath after a 25 min equilibration period were 620 ± 30, 88 ± 1 and 8 ± 1 mmHg respectively for the gas mixtures of 95% $O_2$: 5% $CO_2$, 12% $O_2$: 5% $CO_2$ in $N_2$, and 95% $N_2$: 5% $CO_2$. For all the gas mixtures, the pH of the solution was 7.50 ± 0.06 and the $Pco_2$ was 34 ± 3 mmHg.

*Effects of pharmacological antagonists on the hypoxic contraction:* After the control hypoxic challenge, the artery was allowed to return to baseline tension before an antagonist (phentolamine and propranolol, indomethacin, verapamil or the solvent absolute ethanol) was added and allowed to equilibrate for 30 min. Resting tension was adjusted to its optimal value and the hypoxic challenge repeated in the continuing presence of the antagonist. The tension developed under hypoxia before and after the addition of each agent was compared. The experiments with antagonists were performed on the large coronary artery rings only.

*Effects of hypoxia (changing from 95% $O_2$ to 95% $N_2$) on the responsiveness of the coronary artery:* Cumulative concentration-response curves to each agent (under optimal resting tension: KCl, U46619, 5-HT and acetylcholine; in 40 mM KCl precontracted rings: noradrenaline, iloprost and adenosine) were constructed before and after lower-

ing the $Po_2$. The $Po_2$ was reduced for 45 min before a second curve was constructed. No significant further fall in $Po_2$ in the organ bath occurred after 15 min bubbling with 95% $N_2$: 5% $CO_2$. In the presence of hypoxia, each preparation was reset to its optimum resting tension before commencing the concentration-response curve to the agent. A concurrent time-matched control was set up during the noradrenaline, iloprost and adenosine experiments. The percentage change in tension, if any, observed in the response of the parallel control tissue was used to correct the response recorded in the artery rings receiving a vasodilator.

*Effects of different oxygen tensions on the responsiveness of the coronary artery to U46619:* Cumulative concentration-response curves to U46619 were constructed at different oxygen tensions i.e. in the presence of 95% $O_2$, 12% $O_2$ and 0% $O_2$. When switching from one gas mixture to another, the artery rings were allowed to equilibrate for an hour and tension was readjusted to optimal resting tension before the construction of the second concentration-response curve to U46619.

*Drugs*

Adenosine, 5-hydroxytryptamine creatinine sulphate complex, (±) propranolol hydrochloride, acetylcholine chloride and verapamil (all dissolved in distilled water), noradrenaline bitartrate (NA, dissolved in acidic saline), indomethacin (dissolved in absolute ethanol) (all obtained from Sigma), phentolamine mesylate (Ciba), U46619 (11α,9α-epoxymethano $PGH_2$, dissolved in absolute ethanol and further diluted with distilled water, Upjohn), iloprost (gift from Schering). Vehicles were tested at equal volumes.

*Data analysis*

Results are expressed as mean ± s.e.mean. The $n$ value quoted is the number of hearts used. Significance tests were performed by Student's paired $t$ test. $P$ values of less than 0.05 were considered significant. The $EC_{50}$ values were calculated by using a least squares curve fitting programme with concentrations of drug expressed as log concentrations. The maximum effect of $K^+$, U46619, 5-HT and ACh is expressed as force developed per cross sectional area while for NA, iloprost and adenosine it is expressed as % inhibition of 40 mM KCl-induced contraction.

## Results

*Mechanical effects of hypoxia*

Under optimum resting tension, introduction of hypoxia by exchanging the oxygenated Krebs-Henseleit solution (bubbled with 95% $O_2$: 5% $CO_2$) with the pre-equilibrated hypoxic Krebs-Henseleit solution and bubbling vigorously with 95% $N_2$: 5% $CO_2$, caused an increase in the baseline tension of both large and small coronary arteries. This contraction was reversible on washing with oxygenated Krebs-Henseleit solution. Under oxygenated conditions, 40 mM KCl produced about 85% of its own maximum contraction. The hypoxic contraction was 25–33% of the tension developed by 40 mM KCl. This hypoxic contraction could be replicated at least four times in each artery. In 10 preparations, the four consecutive hypoxic-induced contractions, expressed as a % of the tension developed by 40 mM KCl, were 30 ± 4, 28 ± 4, 32 ± 2 and 31 ± 3%. An increase in tension was also observed on lowering the $Po_2$ from 620 to 88 or from 88 to 8 mmHg; the respective contractions being 18 ± 3% ($n = 8$) and 49 ± 2% ($n = 8$) of that induced by 40 mM KCl.

In coronary arteries precontracted with 40 mM KCl ($n = 5$, large artery; $n = 2$, small artery) hypoxia (introduced by switching from 95% $O_2$: 5% $CO_2$ to 95% $N_2$: 5% $CO_2$, without changing the Krebs-Henseleit solution) caused a transient relaxation followed by a further contraction to reach a new steady-state tension.

*Effects of phentolamine plus propranolol, indomethacin and verapamil on the hypoxic contraction*

Figure 1 illustrates the effects of phentolamine (1 μM) plus propranolol (1 μM), indomethacin (1 μM) and verapamil (10 μM) on baseline tension and on tension development during hypoxia. The hypoxic contraction was potentiated by the combination of phentolamine and propranolol, and markedly inhibited by verapamil. Indomethacin under oxygenated conditions caused an increase in baseline tension. However, under hypoxic conditions, two different phenomena were obtained: in early experiments indomethacin abolished the hypoxic-induced contraction and a relaxation below baseline was obtained (Figure 1). Recently, in the presence of indomethacin, the hypoxic contraction was significantly reduced (from 18.6 ± 3.4 to 11.2 ± 1.7 g cm$^{-2}$; $n = 12$) but not abolished. The baseline tension before the introduction of hypoxia was unaffected by phentolamine plus propranolol or by verapamil. Ethanol (2 μl 10 ml$^{-1}$), the solvent for indomethacin, had no effect on the baseline tension or the hypoxic contraction.

*Effects of hypoxia on the pharmacological responsiveness of the sheep coronary artery*

In control experiments, two replica concentration-response curves for each agonist were constructed

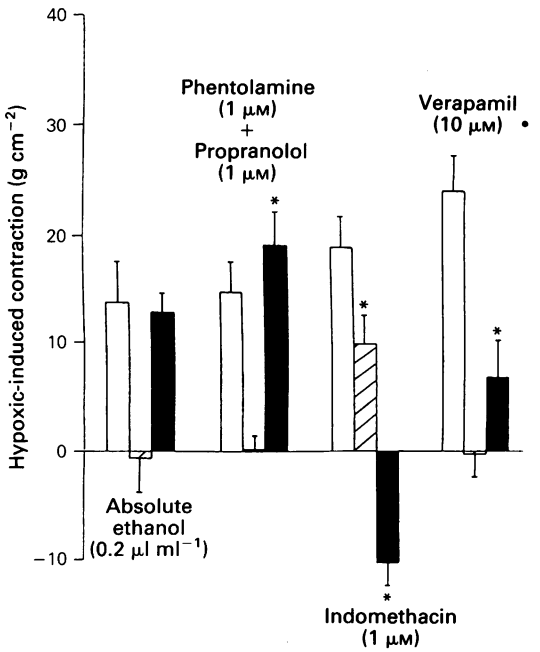


**Figure 1** The hypoxia-induced contraction before (open columns, control) and during (solid columns) the action of various antagonists in left circumflex coronary artery rings isolated from sheep. The effects of the antagonists alone on baseline tension are also shown (hatched column). Vertical lines show s.e.mean. * Denotes significant difference from control at $P < 0.05$.

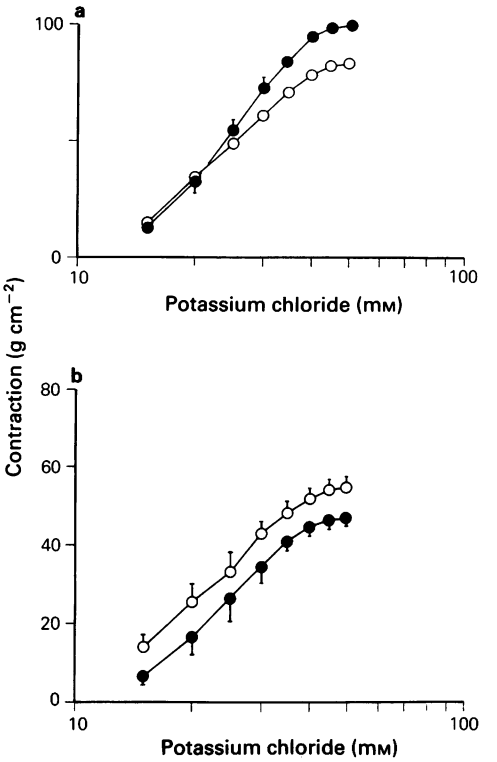


**Figure 2** Concentration-response curves for potassium chloride on left circumflex coronary artery rings isolated from sheep and studied under oxygenated (●) and hypoxic (○) conditions. (a) and (b) Show the results obtained on large and small coronary arteries, respectively. Each point respresents the mean and vertical lines show s.e.mean. ($n = 10$).

under oxygenated conditions in a minimum of four preparations. In each case the second concentration-response curve was not significantly different from the first. The only exception to this was observed with 5-hydroxytryptamine in the small coronary artery. In this case, a severe desensitization to the drug was observed and no consistent second concentration-response curve could be obtained ($n = 4$). Thus, the effects of hypoxia on the responsiveness to 5-hydroxytryptamine of the small coronary artery could not be tested.

Table 1 summarizes the $EC_{50}$ values and the maximum contraction or relaxation observed with the drugs under oxygenated and hypoxic conditions in the large coronary arteries. In both sizes of artery, hypoxia did not modify the $EC_{50}$ of potassium. Hypoxia decreased the maximum tension developed in the large but increased the maximum tension developed by $K^+$ in the small arteries (Figure 2).

The thromboxane $A_2$ stable analogue, U46619, caused a concentration-dependent vasoconstriction under oxygenated and hypoxic conditions. In the large arteries, there was a marked increase in the maximum active tension development and a decrease in the $EC_{50}$ of this agonist during hypoxia (Figure 3). A similar effect was observed in the small arteries; the $EC_{50}$ (μM) being reduced from $0.26 \pm 0.04$ to $0.08 \pm 0.02$ and the maximum effect increased from $50.6 \pm 19.5$ to $102.3 \pm 11.6\,g\,cm^{-2}$. Changing the gas from 95% $O_2$: 5% $CO_2$ to 12% $O_2$: 5% $CO_2$ in $N_2$ did not alter the contractile effect of U46619. However, a change from 12% $O_2$: 5% $CO_2$ in $N_2$ to 95% $N_2$: 5% $CO_2$ significantly enhanced the maximum tension development by U46619 ($63.08 \pm 6.44$ cf $23.04 \pm 5.05\,g\,cm^{-2}$) and decreased the $EC_{50}$ value ($0.08 \pm 0.01$ cf $0.15 \pm 0.02$ μM).

Both 5-hydroxytryptamine and acetylcholine, under oxygenated conditions, caused a biphasic contraction, comprising a phasic component (peaking at 20–25 s) followed by a smaller tonic component (the sizes of the phasic and tonic components are given in Table 1). In the presence of hypoxia, the biphasic

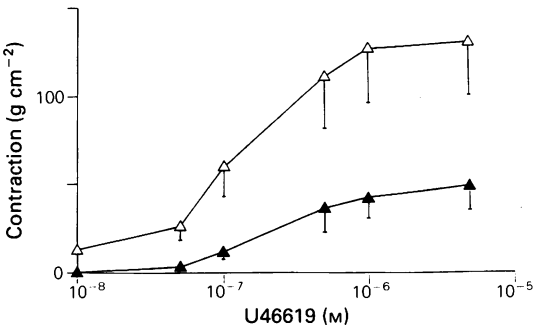


**Figure 3** Concentration-response curves for U46619 on left circumflex coronary artery rings (large) isolated from sheep and studied under oxygenated (▲) and hypoxic (△) conditions. Each point represents the mean and vertical lines show s.e.mean. ($n$ = 8).

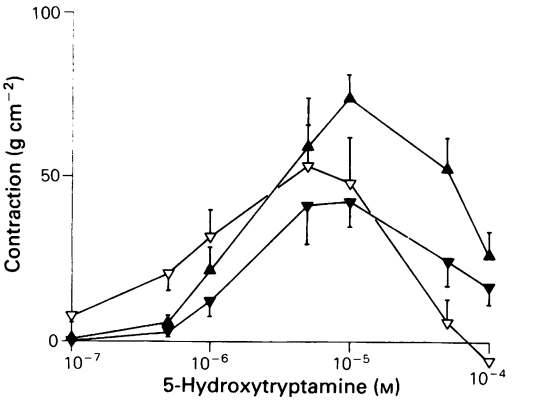


**Figure 4** Concentration-response curves for 5-hydroxytryptamine on left circumflex coronary artery rings (large) isolated from sheep and studied under oxygenated (phasic contraction (▲), tonic contraction (▼)) and hypoxic (▽) conditions. Each point represents the mean and vertical lines show s.e.mean. ($n$ = 8).

contraction to acetylcholine remained whereas 5-hydroxytryptamine elicited only a monophasic tonic contraction. By comparison with the tonic contraction observed under oxygenated conditions, there was a shift to the left and an increase in the maximum tension developed by 5-hydroxytryptamine during hypoxia (Figure 4). In contrast to the potentiation by hypoxia of the contractile effects of U46619 and 5-hydroxytryptamine, the maximum active tension development of acetylcholine was decreased and there was no change in its $EC_{50}$ during hypoxia (Table 1).

In preliminary experiments it was shown that noradrenaline (0.01–10 μM) had no contractile effect on the large coronary arteries both under resting tension ($n$ = 4) and precontracted ($n$ = 2). However, noradrenaline did produce a concentration-dependent relaxation of the large coronary artery precontracted with 40 mM KCl under both oxygenated and hypoxic conditions. The vasorelaxant effect of noradrenaline was augmented significantly by hypoxia giving a decrease in the $EC_{50}$ and an increase in the % inhibition of contraction (Figure 5).

Similar to noradrenaline, iloprost (0.1–10 μM) and adenosine (1 μM–2 mM) caused a concentration-dependent relaxation of the precontracted (by 40 mM KCl) coronary artery. Hypoxia augmented the maximum vasorelaxant effect to both drugs but increased the sensitivity only to iloprost (Table 1). Similarly, in the small arteries, the $EC_{50}$ (mM) for adenosine was unchanged by hypoxia (0.65 ± 0.09 cf

**Table 1** Effects of hypoxia on the responsiveness of large circumflex coronary artery rings obtained from sheep

| | *$EC_{50}$* | | *Maximum effect* (g cm$^{-2}$) | |
|---|---|---|---|---|
| | *Oxygenated* | *Hypoxic challenge* | *Oxygenated* | *Hypoxic challenge* |
| $K^+$ (mM) | 23.90 ± 1.10 | 23.40 ± 0.90 | 81.30 ± 5.80 | 66.40 ± 2.10* |
| U46619 (μM) | 0.30 ± 0.03 | 0.19 ± 0.05* | 48.60 ± 14.00 | 129.80 ± 29.9* |
| 5-HT (μM) | | | | |
| (P) | 2.60 ± 0.66 | — | 74.09 ± 14.52 | — |
| (T) | 2.95 ± 0.86 | 0.78 ± 0.14* | 42.60 ± 7.80 | 48.30 ± 13.80* |
| ACh (μM) | | | | |
| (P) | 0.78 ± 0.22 | 0.73 ± 0.26 | 76.04 ± 22.62 | 43.08 ± 17.37* |
| (T) | 1.00 ± 0.30 | 0.61 ± 0.26 | 58.36 ± 20.33 | 24.69 ± 12.28* |
| NA (μM) | 2.13 ± 0.31 | 0.65 ± 0.09* | 48.48 ± 3.68 | 88.19 ± 4.78* |
| Ilo (μM) | 1.05 ± 0.20 | 0.44 ± 0.07* | 28.09 ± 1.81 | 86.15 ± 6.06* |
| Adeno (mM) | 0.70 ± 0.10 | 0.60 ± 0.06 | 87.50 ± 8.90 | 154.90 ± 11.60* |

Results are expressed as means ± s.e.mean from 6–10 preparations.
* Indicates a significant difference from the value obtained under oxygenated conditions. (P) and (T) relate to the phasic and tonic contraction respectively. Ilo: iloprost; Adeno: adenosine.

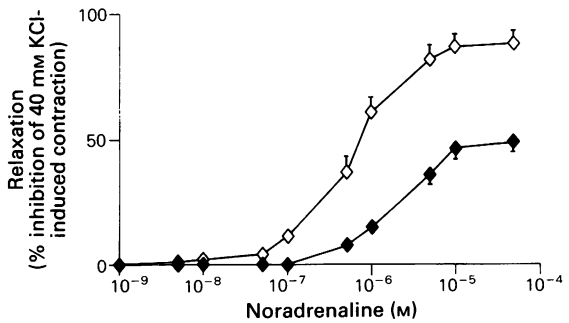


**Figure 5** Concentration-response curves for noradrenaline on precontracted (by 40 mM KCl) left circumflex coronary artery rings (large) isolated from sheep and studied under oxygenated (◆) and hypoxic (◇) conditions. Each point represents the mean and vertical lines show s.e.mean. ($n = 6$).

$0.58 \pm 0.09$) but the maximum relaxation increased from $97.5 \pm 10$ to $203.1 \pm 7.5$.

## Discussion

Lowering the $Po_2$ of the bathing fluid from approximately 620 mmHg to 8 mmHg, cuased a sustained contraction of coronary artery rings isolated from sheep, when the rings were under resting tension. In precontracted rings, a transient relaxation followed by sustained contraction was observed during hypoxia. This phenomenon is not merely a consequence of a change from the hyperoxia employed in oxygenated Krebs solution, since lowering the $Po_2$ from a more physiological 88 mmHg also resulted in contraction. A similar effect of hypoxia to contract coronary arterial rings *in vitro* has been obtained for other species such as the dog (Borda *et al.*, 1980) and the pig (Rubanyi & Paul, 1985), whereas in cattle (Kalsner, 1976; Roberts *et al.*, 1981) a relaxation is observed. The mechanism(s) underlying this hypoxia-induced contraction have not been elucidated. In the present experiments indomethacin either reduced or abolished contractions caused by hypoxia. This implies that in the coronary artery of the sheep the hypoxic contraction is, in part, mediated either by the release of a vasoconstrictor prostanoid or by a reduced synthesis of a vasodilator prostanoid. The fact that indomethacin, under oxygenated conditions, produced a contraction suggests there is likely to be a basal release of a vasodilator prostanoid from the coronary artery which might be inhibited by hypoxia. In other species conflicting results with indomethacin have been described. In the dog (Rubanyi & Vanhoutte, 1985) indomethacin had no effect on the hypoxic contraction, whereas in porcine isolated arteries (Rubanyi & Paul, 1985) and in Langendorff perfused hearts of the rat (Karmazyn *et al.*, 1979) it abolished vasoconstriction during hypoxia. Therefore, it seems probable that the mediators released from the coronary artery by hypoxia are species-dependent.

Verapamil substantially reduced the hypoxic contraction indicating that part of it is dependent upon the influx of $Ca^{2+}$ from the extracellular fluid, as has been suggested previously by Van Neuten *et al.* (1983) and Karmazyn *et al.* (1984). This dependence on extracellular $Ca^{2+}$ supports the idea that hypoxia may release a vasoconstrictor mediator rather than solely reducing the release of a vasodilator. In canine coronary arteries hypoxia evokes release of noradrenaline which promotes contraction via $\beta$-adrenoceptor activation (Borda *et al.*, 1980). However, our data show that the combination of phentolamine plus propranolol significantly augmented the hypoxic contraction indicating that although hypoxia may release noradrenaline it has a vasodilator action in sheep coronary arteries.

Under oxygenated conditions, coronary artery rings from sheep were contracted by $K^+$, 5-hydroxytryptamine, U46619 (the thromboxane $A_2$ analogue) and acetylcholine but relaxed by noradrenaline, iloprost (a prostacyclin mimetic) and adenosine in a concentration-dependent manner. These results confirm previous findings (Schror *et al.*, 1980; Berne, 1980; Lewy *et al.*, 1981; Perez *et al.*, 1983), mostly in species other than the sheep, that $K^+$, U46619 and 5-hydroxytryptamine are vasoconstrictors whereas iloprost and adenosine are vasorelaxant in the coronary bed. Acetylcholine has been shown to cause an endothelium-dependent relaxation in a variety of isolated arteries (Vanhoutte & Rimele, 1983) but in sheep (Feletou *et al.*, 1986) as well as in the human coronary artery (Kalsner, 1985) it causes contraction. This probably reflects the fact that in these species, acetylcholine has a greater direct effect on the smooth muscle cells than on the endothelium to release endothelium-derived relaxant factor (EDRF). The vasorelaxation observed with noradrenaline is consistent with the observations by Brine *et al.* (1979) that sheep coronary arteries contain few if any $\alpha$-adrenoceptors but that $\beta$-adrenoceptors mediate relaxation.

In the large coronary arteries, hypoxia augmented the contractile effects of U46619 and 5-hydroxytryptamine but depressed those of acetylcholine and $K^+$. The experiments carried out with U46619 under different oxygen tensions indicate that the potentiating effect, at least for this agent, mainly results from a lowering of the $Po_2$ from a physiological to a hypoxic one. The reason for the differential effect of hypoxia on the responsiveness to the various agents studied is not known. One possible explanation is that hypoxia causes a specific change in certain receptors or the intracellular signalling

systems linked to them. On the the other hand, it may be that acetylcholine, like $K^+$, mediates contraction via an influx of $Ca^{2+}$ from the extracellular space whereas U46619 and 5-hydroxytryptamine release $Ca^{2+}$ from intracellular stores. Since hypoxia appears to increase $Ca^{2+}$ influx across the smooth muscle cell membrane it may thereby potentiate the contraction caused by U46619 and 5-hydroxytryptamine but depress the maximum contraction obtained with $K^+$ and acetylcholine.

Hypoxia also augmented the maximum relaxations observed with adenosine, noradrenaline or iloprost but it reduced the $EC_{50}$ values of the latter two drugs only. The interaction between hypoxia and the vasorelaxant effect of adenosine is in agreement with Gellai *et al.* (1973) who demonstrated that the vasodilator effect of adenosine was potentiated under low oxygen tension. The mechanism(s) underlying this potentiation by hypoxia of the vasorelaxant agents has not been studied. A direct effect of hypoxia to inhibit contraction of the smooth muscle can be excluded since under these experimental conditions, it causes contraction. Hypoxia has been shown to block extraneuronal noradrenaline uptake in rat isolated heart (Inoue *et al.*, 1987) which could account for a shift to the left of the noradrenaline concentration-response curve. However, such a hypoxia-induced increase in the local concentration of noradrenaline, or of the other two substances tested, could not explain the increase in the maxium relaxation observed.

In conclusion, in isolated coronary arteries of the sheep, hypoxia caused a contraction and modified the responsiveness to both vasoconstrictor and vasodilator substances that may be released during myocardial ischaemia. These results may have clinical relevance both to the aetiology of coronary vasospasm, the underlying mechanisms of which are unknown, and to the effects of various mediators or drugs on coronary vascular tone during myocardial ischaemia.

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