Two Distinct Effects of Oxygen on Vascular Tone in Isolated Porcine Coronary Arteries

Gabor Rubanyi and Richard J. Paul

From the Department of Physiology, University of Cincinnati, College of Medicine, Cincinnati, Ohio

SUMMARY. The relation between Po2 and vessel tone was studied in isolated porcine left descending coronary artery rings. Porcine left descending coronary artery mounted isometrically and equilibrated in Krebs-bicarbonate solution (37°C, pH 7.4, when gassed with 95% oxygen + 5% carbondioxide) exhibited spontaneous basal tone. Decreasing bath Po2 to 40%, 20%, and 12% elicited sustained increases in basal tension which were reversible, ranging between 10% and 20% of the contraction induced by 40 mM potassium chloride. Further decreases in Po2 to near zero (anoxia) resulted in relaxation to baseline. Cyclooxygenase inhibitors indomethacin (5.5 X 10^-6 M), aspirin (5 X 10^-5 M), and meclofenamate (10^-5 M) decreased vascular tone and totally prevented coronary vasoconstriction induced by lowering bath Po2 to 12% or 40% but did not affect anoxic vasorelaxation. Neither basal tone nor the vasoconstriction induced by decreases in bath Po2 were influenced by the antihistaminergic drug pyribenzamine (10^-5 M) or by the α-adrenergic blocker phentolamine (10^-6). Isoproterenol (10^-8 to 10^-6 M) or an elevation of the bath potassium concentration from 5.9 to 11 mM significantly augmented coronary vasoconstriction induced by lowering bath Po2 from 95% to 40%. Elevation of the bath potassium chloride concentration to 40 mM further increased isometric force but inhibited the vasoconstriction in response to decreasing Po2 from 95% to 40%. Anoxia relaxed contractions induced by 40 mM potassium chloride, histamine, or ouabain. The data suggest the existence of two distinct oxygen-sensitive mechanisms in porcine coronary arteries, both of which regulate vascular tone. One is activated at relative high Po2 values (10-40%), and the vasoconstriction induced by this mechanism is mediated by vascular prostaglandin synthesis. The other is expressed at low Po2 values (near zero), and the depression of mechanical activity by this mechanism may be related to limitation of oxidative energy metabolism. The first mechanism can be augmented by β-adrenoceptor stimulation indicative of an interaction between vascular prostaglandin synthesis and β-adrenergic mechanisms in the coronary artery wall. (Circ Res 56: 1-10, 1985)

It is well documented that mechanical tension in some vascular smooth muscle preparations is sensitive to organ bath oxygen tension (Carrier et al., 1964; Smith and Vane, 1966; Detar and Bohr, 1968; Fay, 1971; Gellai et al., 1973; Chance et al., 1977; Coburn, 1977; Coburn et al., 1979; Shepherd and Vanhoutte, 1979; Needleman and Isakson, 1980; Singer et al., 1981). Pittman and Duling (1973) provided data and a mathematical model which suggested that the oxygen sensitivity of in vitro artery strips is the result of an anoxic core within each strip in the muscle bath. Pittman and Duling’s model is based on the premise that the anoxic core limits oxidative energy generation, which in turn limits the mechanical tension development. Coburn’s experiments in rabbit aortic strips, however, produced evidence which was not consistent with the suggestion that the vascular oxygen sensor is the mitochondrial respiratory chain (Coburn, 1977; Coburn et al., 1979). Mechanical tension was still affected by alteration in organ bath Po2 after inhibition of detectable oxygen uptake by cyanide treatment. It was suggested that there is an oxygen-sensitive system other than electron transport chain which regulates vascular smooth muscle tension. The nature of this oxygen sensitivity is still unknown.

There are various reports throughout the literature to the effect that, in response to changes in oxygen tension, alterations in the rate of prostaglandin (PG) biosynthesis within the vessel wall appear to be determinants of coronary arterial tone (Kalsner, 1975, 1976, 1977; Needleman et al., 1977; Roberts et al., 1981). These studies (though restricted to bovine coronary arteries only) support Coburn’s theory that there is an oxygen sensitivity in the coronary vessel wall which is not related to the respiratory chain, and suggest that it may be linked to vascular prostaglandin synthesis. However, because the responses observed in these studies to decreases in
Po2 were in the direction of vasodilation, a separation of the underlying mechanism(s) is not straightforward. Thus, the existence of an oxygen-sensitive system different from the respiratory chain is yet controversial.

Based on preliminary experiments on the relation between muscle tension and bath Po2 (Rubanyi and Paul, 1983), it appeared that the porcine coronary artery could be ideally suited to demonstrate distinct responses to oxygen. In the present study, the coexistence of two distinct responses to decreases in bath Po2 is described in isolated porcine coronary artery rings. They were identified and separated from each other by the different bath Po2 ranges, by the direction of mechanical responses, and by their differential sensitivity to cyclooxygenase inhibitor drugs.

An unusual and novel characteristic of the response at high Po2 suggested by our results is that it not only involves vascular prostaglandin metabolism, but also involves interaction with β-adrenergic mechanisms. A characterization of this interaction between prostaglandin biosynthesis and β-adrenergic mechanisms suggested that it may underlie the "paradox" reversal of β-adrenergic responses under ischemic conditions (Moore and Parratt, 1973; Barcia et al., 1976; Vatner et al., 1977), and thus play a role in the development of coronary vasospasm.

Methods

Preparation of Arterial Rings

Pig hearts obtained promptly after slaughter were drained of blood and immersed in ice-cold (4°C) modified Krebs-bicarbonate solution. The distal portions of the left anterior descending coronary artery were carefully dissected out (maximum, within 30 minutes after slaughter) and placed in cold Krebs-bicarbonate solution in a Petri dish. Arteries were then cleaned of fat and adhering connective tissue. Care was taken to avoid stretching. Most of the arteries that were used had an outside diameter of 1.5–2.0 mm. The arteries were cut into rings 5 mm long.

Recording of Muscle Tension

Arterial rings were suspended in a water-jacketed muscle chamber filled with 70 ml of Krebs-bicarbonate solution maintained at 37°C and gassed with 95% O2, 5% CO2 from a fritted glass outlet at the bottom of the chamber. Each arterial ring was suspended on a pair of stainless-steel hooks, one of which was fixed to a plate inside the chamber and the other to a Kistler-Morse DSK force transducer. The transducer was attached to a rack and pinion device above the chamber in order to adjust the length of the muscle ring. Tension was measured isometrically and recorded (Linear instrument 585 recorder). The muscle chamber was filled with Krebs-bicarbonate solution at the top of the chamber and washing was accomplished by allowing solutions to drain at the bottom of the chamber.

Recording of Bath O2-Tension

The Po2 of the bathing medium was continuously measured using a Clark-type oxygen electrode (YSI 5331) immersed into the bathing solution and was recorded on the Linear Instrument recorder in parallel with isometric muscle tension. Note that the actual time course of changes in Po2 is shown representatively in Figure 3 only, whereas Figures 1, 2, 4, and 5 schematically show the initial and steady state Po2 values. The average response time to attain 90% of a new steady state Po2 was 3.7 ± 0.3 minutes and to the steady state was 8.2 ± 0.5 minutes.

Equilibration of Arterial Rings

Arterial rings were equilibrated in Krebs-solution gassed with 95% O2, 5% CO2 for 2 hours at their optimal length [at a resting (unstimulated) tension of approximately 100 mN (10 g)]. We selected this load on the basis of preliminary experiments showing that rings equilibrated in this manner responded to high potassium (40 mM) depolarization with maximal active tension development. Since porcine coronary artery (PCA) rings undergo stress relaxation, they were restretched to obtain 100 mN at 30-minute intervals, at which time the chamber was filled with fresh Krebs-bicarbonate solution. Stress relaxation usually decreased within 1 hour.

Solutions

The modified mammalian Krebs-bicarbonate solution used for preparation of tissues and as the bathing medium had the following composition (mM): NaCl (118), KCl (4.7), CaCl2·H2O (2.5), KH2PO4 (1.18), MgSO4 (1.18), glucose (5.5), and NaHCO3 (25). All the constituents (Sigma) were dissolved in distilled, demineralized water. Increasing extracellular K+ concentration was carried out by adding KCl from a concentrate (3 m) to the muscle bath.

The bathing medium was gassed with mixtures containing 95% O2, 5% CO2; 40% O2, 55% N2, 5% CO2; 20% O2, 75% N2, 5% CO2; 12% O2, 83% N2, 5% CO2 or 95% N2, 5% CO2, giving a pH of 7.4 in the Krebs-bicarbonate buffer solution at 37°C. When changing from one gas mixture to another, the solution in the muscle chamber was exchanged for one pre-equilibrated with the new gas mixture.

Chemicals

Stock solutions of reagent grade drugs were prepared as follows: acetylsalicyclic acid (ASA; aspirin, dissolved in Krebs solution, 1 mg/ml); histamine (dissolved in H2O, 10−2 M); indomethacin (dissolved in Krebs-solution, 0.2 mg/ml); isoproterenol (dissolved in H2O; 10−4 M); sodium meclofenamate (dissolved in H2O; 10−2 M); sodium nitroprusside (dissolved in H2O; 10−2 M); ouabain octahydrate (dissolved in H2O; 5 × 10−3 M); papaverine HCl (dissolved in H2O; 10−2 M); pyribenzamine (dissolved in H2O, 10−2 M); phenolamine (dissolved in H2O, 10−3 M); l-propranolol (dissolved in H2O, 10−3 M); and d-propranolol (dissolved in H2O, 10−3 M). Drugs were added to the muscle bath in aliquots up to 700 μl.

Calculations

Muscle contraction was measured as the increase in tension above the initial baseline. Responses were calculated as active force development related to cross-section of the vascular ring (mN/mm²), or expressed as the percent of the 40 mM KCl-induced test contraction. At the end of an experiment, the rings were cut open and the length (l) and the width, as well as the blotted weight (blot wt), were measured. Vessel cross-section area was calculated.
Rubanyi and Paul/Oxygen and Coronary Vascular Tone

as 2 x blot wt/1.06 (Paul, 1983). Data are reported as mean values with standard errors of the mean. Results were analyzed by Student's t-test with \( P < 0.05 \) considered statistically significant.

Results

Characteristics of Isometric Contraction in Porcine Coronary Arteries

During the equilibration period, after the tissue length for optimal force development (see Methods) had been adjusted, in many, but not all arteries (in 26 out of 35), a spontaneous development of force took place. This force is often referred to as "basal tone," which we take to mean that part of the total isometric force at a given length which is attributable to active force generation by the contractile elements in the absence of exogenous stimulation. The spontaneous force which developed during equilibration was variable. In the majority of cases (\( n = 19 \)) it was small, averaging 22.5 ± 3.5% of the contractile force elicited by KG (40 min). However, in an appreciable number of cases (\( n = 7 \)), this spontaneously developed force averaged 69.7 ± 7.7% of a KG contracture. The basis for this difference is unknown. In all cases studied, however, the spontaneous force was abolished by prolonged anoxia, i.e., 2.4 ± 1.2% (\( n = 7 \)) of the spontaneously developed force remained following prolonged (20-30 minutes) exposure to N2. Similarly, isoproterenol (10^-7 M) essentially abolished the spontaneously developed force with 2.5 ± 1.1% (\( n = 10 \)) remaining following exposure. Similar results were obtained with papaverine (10^-4 M) or sodium-nitroprusside (10^-4 M). Because of these characteristics consistent with the definition given above, the spontaneously developed force will be referred to as basal tone.

The isometric force generated by 40 mM KG above the existing basal tone was 39.6 ± 3.4 mN/mm² (\( n = 29 \)), which is consistent with previously published values (Paul, 1983). It should be noted that other agonists can elicit up to twice this active force (Paul et al., 1979). Thus, basal tone is only in the order of 10% of the maximum isometric force. It is also noteworthy that the level of basal tone was only weakly correlated with the force elicited by KCl (\( r = 0.48 ; \ P < 0.05 ; n = 19 \)).

Responses of Porcine Coronary Artery to Decreases in Bath Po2

Anoxia

Figure 1 depicts the typical responses of porcine coronary arterial rings to alteration in O2 tension (Po2) of the Krebs-bicarbonate solution. When the Po2 of the bathing medium was decreased by changing the gas mixture from 95% O2 to 95% N2 (anoxia), tension of the PCA ring increased transiently (phase 1). After approximately 5 minutes, tension began to fall (phase 2) and reached its lowest level in about 20 minutes (not shown in the figure, see Fig. 2). Similarly, after an initial transient augmentation, anoxia abolished contractions evoked by histamine (10^-4 M), ouabain (10^-5 M), or high K+ (40 mM) (data not shown, five tissues studied for each case). Reoxygenation with 95% O2 during phase 1 or shortly after the onset of phase 2 (i.e., before total relaxation of the ring), resulted in a brief relaxation (phase I) followed by contraction (phase II) to levels higher than the original basal tone and, finally, by relaxation to the original tone. The mean value of the peak vasoconstriction elicited by anoxia in 13 PCA rings in the absence of exogenous stimulation was 9.9 ± 1.5% (\( P < 0.01 \)) of the control contraction induced by 40 mM KCl.

Hypoxia

When the Po2 of the bathing medium was decreased by changing the gas mixture from 95% O2 to 12% O2 (hypoxia), tension of PCA rings increased as well (Fig. 1), but in contrast to anoxia, hypoxia induced a sustained contraction. Only when the gas mixture was returned back to 95% O2 did the ring relax to the initial level of basal tone. The mean value of the hypoxia-induced vasoconstriction of 10 PCA rings was 11.3 ± 1.2% (\( P < 0.01 \)) of the control contraction induced by 40 mM KCl.

![Figure 1](http://example.com/figure1.png)

**Figure 1.** Responses of porcine coronary artery rings to changes in bath Po2. Reduction in oxygen tension by gassing the bathing medium with 95% N2, 5% CO2 (anoxia) induced a transient contraction (phase 1) followed by relaxation (phase 2). Reoxygenation by 95% O2, 5% CO2 resulted in a relaxation (phase II) followed by contraction (phase I) above baseline. The decrease of bath Po2 to 12% (hypoxia) or 40% elicited a sustained contraction (10-15% of 40 mM KCl-induced test contracture, left hand side of the figure), which was reversible upon reoxygenation with 95% O2.
Decrease in Bath $P_{O_2}$ from 95% to 40%

To examine whether the transient (anoxia) or sustained (hypoxia) contraction of PCA rings induced by varying bath $P_{O_2}$ was related to mitochondrial respiration limited by oxygen, we studied the effect on basal coronary tone of decreasing bath $P_{O_2}$ from 95% to 40%. PCA rings responded to a decrease in bath $P_{O_2}$ from 95% to 40% with a sustained contraction (Fig. 1). When the gas mixture was changed back to 95% $O_2$, the rings relaxed and reached the level of tone observed prior to the decrease in $P_{O_2}$ to 40%. The average value of coronary vasoconstriction induced by the $P_{O_2}$ change in 10 PCA rings was 14.9 ± 2.3% ($P < 0.01$) of the $KCl$-induced control contraction. These results were similar to the initial phase observed in the response to anoxia and the response to hypoxia. It is of interest to note that the magnitude of the response to a decrease in $P_{O_2}$ was not correlated with the level of basal tone measured prior to the decrease in $P_{O_2}$ ($r = 0.22; NS; n = 24$).

Effect of Cyclooxygenase, Adrenergic, and Histaminergic Inhibition on the Changes in Vascular Tone Induced by a Decrease of Bath $P_{O_2}$

In five experiments, four rings cut from the same coronary artery were suspended in individual muscle baths. After $P_{O_2}$ cycles of 95-40-95% and 95-0-95% (anoxia), rings number 2, 3, and 4 were treated with the cyclooxygenase inhibitors, indomethacin ($5.5 \times 10^{-6} M$), aspirin ($5 \times 10^{-5} M$), or meclofenamate ($10^{-5} M$), respectively, while the first ring served as control. As shown in a typical recording in Figure 2, all of the cyclooxygenase inhibitors decreased basal tone (by approximately 50%) and totally abolished the sustained vasoconstrictor response induced by a decrease in bath $P_{O_2}$ from 95 to 40%, and the initial transient increase in tone during anoxia. None of the inhibitor drugs affected the vasorelaxation to baseline induced by prolonged anoxia (Fig. 2). Although basal tone was reduced by these agents, the complete inhibition of the vasoconstrictor response to decreasing bath $P_{O_2}$ cannot be ascribed to a generalized inhibition of contractility since, in the presence of these agents, the magnitude of a $KCl$ contracture was unaffected. Moreover, the restoration of tension to the previous basal tone levels by an elevation of bath $K^+$ concentration from 5.9 to 12.9 mM did not restore the vasoconstriction induced by decreasing bath $P_{O_2}$ (Fig. 2).

To investigate the possible role of the release of other vasoconstrictor substances in the hypoxic mechanical response, we tested the effect of histamine and catecholamine inhibitors in five PCA rings. As shown in a typical recording in Figure 3, neither basal tone nor the reversible vasoconstrictor response to the change from 95% to 40% $O_2$ in the PCA strip was influenced by the antihistamine ($H_1$-receptor antagonist) pyribenzamine ($10^{-5} M$), or by the $\alpha$-adrenergic inhibitor, phentolamine ($10^{-6} M$).

Isoproterenol and Increasing Extracellular $K^+$ Concentration Alter Coronary Vasoconstriction Induced by a Decrease in Bath $P_{O_2}$ from 95 to 40%

Treatment of PCA rings with increasing doses (3 $\times$ $10^{-9}$ to $10^{-8} M$) of isoproterenol (ISO) relaxed basal tension progressively and significantly augmented the vasoconstriction induced by decreasing bath $P_{O_2}$. The $P_{O_2}$ response was depressed at a higher isoproterenol concentration ($10^{-7} M$) (Fig. 4). The potentiating effect of $10^{-8} M$ isoproterenol was statistically significant both when expressed as percent of control (Fig. 7) or as the absolute isometric force increment (Table 1).

Elevation of the $K^+$ concentration in the Krebs-bicarbonate solution from 5.9 to 11 mM increased isometric force, which reached a maximum approximately 20 minutes after the addition of $KCl$. The vasoconstrictor response of the PCA ring evoked by decreasing the bath $P_{O_2}$ to near 0% (anoxia), 10%
Rubanyi and Paul/Oxygen and Coronary Vascular Tone

FIGURE 3. Effect of histamine and adrenergic-receptor antagonists on mechanical responses of a porcine coronary artery ring to a decrease in bath PO₂ from 95 to 40% O₂.

Pyribenzamine (histamine-antagonist) and phentolamine (α-adrenergic antagonist) did not influence basal tone or vasoconstriction induced by a decrease of bath PO₂.

FIGURE 4. Typical responses of an isolated porcine coronary artery ring to a decrease in bath PO₂ from 95 to 40% O₂ in the absence and presence of increasing concentrations (3 x 10⁻⁷ to 10⁻⁵ M) of isoproterenol.

(hypoxia), and 40% was significantly higher under this condition (Fig. 5). Elevation of extracellular K⁺ concentration to 40 mM caused a rapid contracture of the PCA ring. The response of the PCA ring to decreasing the bath PO₂ to 40%, 10%, and 0% in the presence of 40 mM K⁺ was significantly diminished when compared to the response in the presence of 5.9 or 11 mM K⁺. It should be noted that this diminished response is not due to a saturation of the absolute isometric force-generating capacity by 40 mM K⁺. Histamine (10⁻⁴ M) for example, can substantially increase isometric force (+32.4 ± 2.9%; n = 5) when added to a 40 mM K⁺-induced contracture. The mean values of the responses to a decrease in bath PO₂ from 95% to 40% of 10 PCA rings (expressed as percent of the control response or as the absolute force increment) in the presence of 11 mM K⁺ and in the presence of 40 mM K⁺ are summarized in Figure 7 and Table 1, respectively.

Addition of 10⁻⁷ M isoproterenol to the muscle bath in the presence of 40 mM K⁺ relaxed the PCA ring (Fig. 6A). When relaxation attained a steady state (~ 20 minutes), the decrease in bath PO₂ from 95% to 40% was repeated. The vasoconstrictor response of the PCA ring to decreasing the bath PO₂ from 95% to 40% O₂ in the presence of 40 mM K⁺ was significantly increased by isoproterenol (Fig. 6A). The mean value of the response of 10 PCA rings in the presence of 10⁻⁷ M isoproterenol and 40 mM K⁺ are summarized in Figure 7 and Table 1.

In order to study the possible role of endogenous norepinephrine release on the increased response to a decrease in bath PO₂ from 95 to 40% elicited by elevation of bath K⁺ concentration to 11 mM, we examined the effect of β-adrenergic receptor blockade by l-propranolol. Treatment of PCA rings with l-propranolol (10⁻⁶ M) caused partial relaxation of a contraction induced by 11 mM K⁺ and, in addition, significantly decreased coronary vasoconstriction induced by the reduction of bath PO₂ (Fig. 6B). A similar concentration of d-propranolol was ineffective (Fig. 7). The mean values of the responses of five PCA rings are summarized in Figure 7 and Table 1.
TABLE 1
Effect of [K+]o, Isoproterenol and α-Prorpanolol on Isometric Force and Vasoconstriction in Response to a Decrease in Bath P02 from 95 to 40% (Δforce) in Isolated Porcine Coronary Artery Rings

<table>
<thead>
<tr>
<th>Experimental conditions</th>
<th>Force (95% O2) (mN/mm²)</th>
<th>ΔForce (95-40% O2) (mN/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.9 mM K+ (basal)</td>
<td>31.7 ± 5.5 (15)</td>
<td>+6.7 ± 0.9* (8)</td>
</tr>
<tr>
<td>5.9 mM K+ + isoproterenol (10^-6 M)</td>
<td>20.2 ± 3.5* (5)</td>
<td>+7.5 ± 1.1† (5)</td>
</tr>
<tr>
<td>11 mM K+ + α-Prorpanolol (10^-6 M)</td>
<td>34.2 ± 4.3 (5)</td>
<td>+4.2 ± 0.8 (5)</td>
</tr>
<tr>
<td>40 mM K+ + isoproterenol (10^-7 M)</td>
<td>40.5 ± 3.6 (5)</td>
<td>+8.1 ± 1.4† (5)</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± SEM; numbers in parentheses represent numbers of experiments.
* Statistically significant difference (P < 0.05) from control (5.9 mM K+).† Statistically significant difference (P < 0.05) from Δforce obtained in the presence of 5.9 mM K+.

Discussion
Coexistence of Two Distinct Oxygen Tension Responses in Porcine Coronary Arteries

In the present study, the coexistence of two distinct oxygen-sensitive responses was described in the isolated porcine coronary artery. If the P02 of the bathing solutions was decreased to near zero, the active mechanical tension developed under basal conditions (basal tone) or when stimulated by exogenous agents was relaxed. This vasorelaxation was not sensitive to cyclooxygenase inhibition, indicating it is unlikely that the effect of low P02 on vascular tone was mediated by vasodilator prostaglandins. The most probable explanation for this phenomenon is the "classical" mechanism which is coupled to the mitochondrial respiratory chain. According to this theory, the inhibition of mechanical tension development below a critical bath P02 level is the result of the development of an anoxic core in the preparation, which limits oxidative energy generation. Although ATP production via glycolysis continues under anoxic conditions in this tissue (Paul et al., 1979), Paul (1983) has shown recently that it provides energy primarily for active ion transport processes across the plasma membrane, and isometric force development is dependent on oxidative energy production in isolated porcine coronary artery rings. An alternative explanation is that anoxia-induced relaxation may be due to a stimulation of ouabain-sensitive electrogenic Na-K pump, as suggested by Detar (1980). This can be excluded, in so far as a decrease in bath P02 to near zero relaxed porcine coronary artery rings preincubated with 10^-7 M ouabain (Rubanyi and Paul, unpublished observations).

The present experiments demonstrate the existence of another O2-sensitive response in isolated PCA rings. The presence of this second oxygensensitive response in the vessel wall could be identified and separated from the first by the following characteristics: (1) it affects coronary vascular tone at relatively high P02 levels (well above the critical P02 value, see below), (2) the direction of vascular tone changes was opposite to that observed at low P02 (vasoconstriction vs. vasorelaxation), and (3) the vasoconstriction induced by this oxygen-sensitive system was abolished by cyclooxygenase inhibitors. The sustained contraction observed on reduction of the P02 from 95 to 40% suggests the existence of an oxygen-sensitive system in the porcine coronary vascular wall which is different from the respiratory chain. Whereas the present studies cannot unambiguously rule out the respiratory chain, the observed effects occur in a P02 range that makes this unlikely. In porcine coronary artery stimulated with KCl, both force and the rate of oxygen consumption decline when the bath P02 is decreased below 7.8% (Paul

FIGURE 5. Typical responses of an isolated porcine coronary artery ring to decreasing bath P02 (from 95 to 0 anoxia), 10 (hypoxia), and 40% O2 at various extracellular potassium concentrations (5.9, 11 and 40 mM).
et al., 1979). A value in this range is consistent with the hypothesis that an anoxic core begins to develop at P02 levels below this value, the so-called "critical P02" (Pittman and Duling, 1973; Gluck and Paul, 1977). Though the absolute value of the critical P02 may be subject to some uncertainty, for P02 levels in the range between 40% and 100%, the presence of anoxic regions is highly unlikely. In addition, tissue P02 can be calculated to be substantially higher than that reported to be rate limiting for mitochondrial respiration (Chance et al., 1977). Our present data support Coburn's hypothesis (Coburn, 1977; Coburn et al., 1979) and the observations of Hellstrand et al. (1977) that there is an oxygen-sensitive system other than the electron transport chain in the vessel wall which regulates vascular smooth muscle tone. Among the previous demonstrations of responses to decreases in P02, there is only one, in which the development of an anoxic core in the vascular tissue can likely be excluded.

Kalsner (1977) observed vasorelaxation and an increased synthesis of prostaglandin-like material in isolated bovine coronary artery strips when P02 was decreased from 515 to 112 mm Hg. However, an increase in vascular tone by changes in P02 in this relatively high range has not been demonstrated.

In order to avoid a hypoxic core in isolated vascular preparations, the vast majority of such in vitro experiments are performed at high, unphysiological oxygen concentrations. The facts that (1) a distinct oxygen-sensitive response could be demonstrated at high O2 concentrations, and (2) no further contractions were observed when P02 was decreased from the physiological 20% to 15 or 10% (data not shown), suggest that under physiological conditions this response is fully activated. Changes in conditions which may affect this oxygen-sensitive system, such as activation of phospholipase A2 (cf. Franson et al., 1979), may thus contribute to the local regulation of coronary arterial tone. These findings at
the same time indicate that suppression of this
mechanism by high O₂ concentrations should be
considered when data obtained in in vitro exper-
iments are related to physiological in vivo condi-
tions. Because the vasoconstriction induced by decreasing PO₂ from 95 to 40% could be totally prevented by indomethacin, aspirin, and meclofenamate, we suggest that this oxygen-sensitive response is related to vascular prostaglandin (PG) biosynthesis via the cyclooxygenase pathway. This is supported by the similar effects of these different cyclooxygenase in-
hibitors. The final experimental demonstration of this hypothesis, however, must await the chemical determination of endogenous arachidonic acid metab-
ilites in the coronary arteries during changes in PO₂.

Previous studies on isolated bovine coronary ar-
tery preparations revealed that continuous synthesis and release of PGs (most probably PG1₂) contributes to the regulation of vascular tone in this vessel (Kalsner, 1975, 1976, 1977; Needleman et al., 1977; Needleman and Isakson, 1980; Roberts et al., 1981). These studies on only one (bovine) preparation lead to the generalized view that local production of PGs in coronary vascular tissue causes vasodilation (Needleman and Isakson, 1980) and inhibition or alteration of the prostaglandin release mechanism (by anoxia under in vitro conditions) was implicated in the etiology of coronary vasospasm (Kalsner, 1977; Needleman and Isakson, 1980).

Our data show that—in contrast to the results for bovine coronary artery—in porcine coronary rings, inhibition of PG synthesis leads to relaxation of basal tone, suggesting that a continuous release of vasoconstrictor cyclooxygenase product(s) contributes to maintenance of basal tone in PCA. In addition, our data suggest that a decrease in bath PO₂ could lead to an increased synthesis of this product resulting in vasoconstriction, as the vasoconstrictor response to PO₂ decreases could be prevented by indomethacin, aspirin, or meclofenamate. Although both basal tone and the vasoconstrictor response to a decrease in PO₂ were diminished by these PG synthesis inhibitors, control experiments indicated that this was not a reflection of a nonspecific inhibi-
tion of contractility. Neither initial basal tone nor the changes in basal tone induced by the antagonists were correlated with the responses to decreases in PO₂. Furthermore, the possibility that the cyclooxy-
genase inhibitors prevented coronary vasoconstric-
tion due to a general reduction in force can be
excluded because (1) restoration of force to the pre-
vious levels of basal tone in the presence of indomethacin, aspirin or meclofenamate did not restore the vasoconstriction induced by a decrease in PO₂, and (2) both a decrease in basal tone or the force induced by 40 mM KCl by isoproterenol and an increase in force by elevation of extracellular K⁺ concentration (to 11 mM) significantly augmented the isometric force induced by lowering bath PO₂ from 95 to 40%. The present assumption of in situ synthesis and release of vasoconstrictor substance via cyclooxygenase pathway in PCA rings is sub-
stantiated by previous data, which showed that both the labile endoperoxides and the primary prostag-
landin(s) synthesized from them induce vasoconstric-
tion in the porcine coronary artery (Needleman and Isakson, 1980).

Possible Interactions of Vascular Prostaglandin Synthesis and β-Adrenergic Mechanisms in the Coronary Arterial Wall

Pretreatment of PCA rings with phentolamine (α-adrenergic blockade) or with pyribenzamine (which inhibits histamine-induced contracture in this prep-
paration) did not influence coronary vasoconstriction induced by decreasing bath PO₂ from 95% to 40%. However, stimulation of β-adrenoceptors by isopro-

---

**FIGURE 7. Summary of the Effects of β-Adrenergic Receptor Stimulation (Isoproterenol) and β-Blockade (I-Propranolol) on Bath PO₂ Decrease (from 95 to 40% O₂) Induced Vasoconstriction of Isolated Porcine Coronary Arteries in the Presence of Various Extracellular Potassium Concentrations.** The effects are expressed as a percent of the control response. Columns represent the mean of at least five experiments, and bars represent the SEM.
They found that a contraction induced by 25 mM K⁺ inhibition (40 mM K⁺) inhibited the vasoconstriction induced by a reduction in bath Po₂ in PCA rings. However, they had different properties; whereas the contraction induced by 11 mM K⁺ caused a rapid contraction which was insensitive to β-adrenergic receptor blockade. Similar observations were made by Borda et al. (1980) in isolated canine femoral arteries. These results, however, could not be confirmed in recent experiments (Rimele and Vanhoutte, 1984) excluding the involvement of vasocostrictron prostanoids in that response.

The present experiments have shown that an elevation of the bath K⁺ concentration from 5.9 to 11 mM significantly potentiated, but high K⁺ depolarization (40 mM K⁺) inhibited the vasocostrictron induction by a reduction in bath Po₂ in PCA rings. Similar observations were made by DeMey and Vanhoutte (1983) on isolated canine femoral arteries. They found that a contraction induced by 25 mM K⁺ was significantly augmented by hypoxia but responses to 50 mM K⁺ were not. Both 11 and 40 mM K⁺ caused an increase of vascular tone in PCA rings, but they had different properties; whereas the contraction induced by 11 mM K⁺ developed slowly and could be partially inhibited by I-propranolol, 40 mM K⁺ induced a rapid contraction which was insensitive to β-adrenergic receptor blockade. Similar observations were made by Borda et al. (1980) in the isolated canine coronary artery. They also showed that elevation of external K⁺ concentration up to 12–15 mM stimulated the release of endogenous norepinephrine (³H-NE), but higher concentrations (above 20 mM) inhibited ³H-NE release. These authors suggested that coronary contraction elicited by an increase of K⁺ up to 15 mM is mediated by the release of NE from adrenergic nerves, since it was accompanied by increase of ³H-NE release and could be inhibited by denervation or β-adrenergic receptor blockade (Borda et al., 1977, 1980).

The present finding of a potentiation of the vasoconstrictor response to decreases in Po₂ induced by β-adrenergic receptor stimulation and these earlier observations indicate that the potentiation of hypoxic vasocostrictron in PCA rings by elevation of K⁺ up to 12 mM is likely due to the release of endogenous NE and consequent stimulation of β-adrenergic receptors. This hypothesis is strengthened by the observation that both the vasocostrictron and potentiation of the oxygen sensitive response by 11 mM K⁺ were significantly depressed by I-propranolol but not by d-propranolol in PCA rings. These data, in conclusion with the observations that, in open-chest animals subjected to coronary ligation, the potassium concentration in blood samples collected from venules in the ischemic zone may exceed 10 mM (Benzing et al., 1971; Downar et al., 1977; Schuchleib et al., 1976) indicate a potential role of hypoxic-ischemic K⁺ release from cardiac and coronary vascular cells in the pathogenesis of coronary vasospasm.

The inhibition of coronary vasocostrictron induced by a reduction in bath Po₂ from 95 to 40% by higher K⁺, on the other hand, may be explained by three classes of mechanisms: (1) Inhibition of endogenous NE release (as demonstrated by Borda et al., 1980) may decrease β-adrenergic tone, and similar to β-adrenergic blockade by I-propranolol can decrease this vasocostrictron response to Po₂ changes from 95 to 40% by eliminating the potentiation exerted by β-adrenergic mechanisms. (2) Depolarization of the vascular cell membrane by high K⁺ may directly inhibit the synthesis and/or the vascular effect of the vasocostrictron PG(s). (3) The cellular mechanism(s) involved in this Po₂ response may require a certain level of cell membrane polarization. The present finding that administration of isoproterenol to high K⁺-treated coronary arteries can restore oxygen sensitivity supports the first class of mechanisms. However, these classes are not mutually exclusive, and further studies are needed to resolve the exact nature of high K⁺-induced inhibition of this Po₂ response in isolated porcine coronary arteries.

The demonstration that this vascular PG synthesis mediates coronary vasocostrictron induced by a decrease of bath Po₂ and this vasocostrictron can be potentiated by β-adrenergic agonists may indicate that β-adrenergic mechanisms modulate the intrinsic PG-biosynthesis and/or the vascular action of PGs in coronary arteries. Although the exact nature of this interaction is not known, we suggest that under
null
Two distinct effects of oxygen on vascular tone in isolated porcine coronary arteries.
G Rubanyi and R J Paul

Circ Res. 1985;56:1-10
doi: 10.1161/01.RES.56.1.1

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/56/1/1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/