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# CONTRACTION OF HUMAN UMBILICAL ARTERY, BUT NOT VEIN, BY OXYGEN

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SUMMARY

1. The $O_2$ tension of umbilical arterial blood *in utero* is 15 mmHg. The contractile effect of increasing the $O_2$ tension above this value was studied quantitatively *in vitro* in preparations of human umbilical artery and vein.

2. The umbilical arterial smooth muscle was contracted in a concentration-related manner by stepped increments in $O_2$ tension. The threshold for $O_2$-induced contraction was estimated to be 36 mmHg at pH 7·28 and the maximum contraction occurred at 297 mmHg.

3. The sensitivity of the preparations to low $O_2$ tensions (< 100 mmHg) was reduced by increasing the pH of the bathing medium from 7·28 to 7·36, at which the threshold was 69 mmHg and the maximum contraction occurred at 282 mmHg. Reducing the pH to 7·18 did not significantly change the sensitivity from that found at pH 7·28.

4. Indomethacin (0·1 $\mu$M) virtually abolished responses to $O_2$.

5. The results indicate that the increase in the physiological $O_2$ tension at birth, from 15 to 100 mmHg, may be an adequate stimulus to effect the closure of the umbilical artery.

6. In the physiological range $O_2$ did not contract the umbilical venous smooth muscle. This may allow the transfusion of blood from the placenta to the fetus at birth when the $O_2$ tension of umbilical cord blood increases after the onset of breathing.

## INTRODUCTION

$O_2$, at high tensions, has been noted to cause vasoconstriction of human umbilical artery. Bor & Guntheroth (1970), Roach (1972) and Oberhänsli-Weiss, Heymann, Rudolph & Melmon (1972) found that an increase of $O_2$ tension from 'hypoxic' (which is normal for umbilical cord blood) to 'hyperoxic' conditions contracted the arterial smooth muscle. These studies could not answer the question as to whether the increase of $O_2$ tension of umbilical cord blood from fetal (15 mmHg) to neonatal levels following delivery could be an important stimulus in bringing about vessel closure at birth, since the appropriate range was not investigated. However, if contraction

of the vessel smooth muscle does occur at the appropriate levels of $O_2$ it may represent a vital physiological mechanism promoting the change from fetal to neonatal circulation.

Modanlou, Yeh, Hon & Forsythe (1973) found that the fetal blood pH could change significantly from that during early labour (7·28) to that at delivery (7·16) and that in early neonatal life (7·36). It was therefore of interest to study the sensitivity to $O_2$ of the preparations at different levels of pH.

It has been shown that prostaglandins are involved in the other fetal cardiovascular adjustments at birth (ductus arteriosus constriction and pulmonary vasodilation) and that the post-natal rise in $O_2$ tension is crucial to these events (see Coceani, Olley & Lock, 1980 for a review). The possible involvement of prostaglandins in the $O_2$-induced contraction of human umbilical artery was therefore investigated.

We now report a quantitative study of the influence of $O_2$ on the contraction of human umbilical arteries and veins *in vitro*, at oxygen tensions similar to those found in the vessels *in utero*.

## METHODS

Cords from full-term pregnancies were obtained from the Queen Mother's Hospital immediately following delivery and were transferred to the Institute of Physiology. An attempt was made to minimize activation of the smooth muscle contractile apparatus by excluding excess $O_2$. The cords were transported in ice-cold, de-oxygenated Krebs–bicarbonate saline (composition (mM): NaCl, 119; KCl, 4·7; $MgSO_4$, 1·0; $KH_2PO_4$, 1·2; $CaCl_2$, 2·5; $NaHCO_3$, 25·0; glucose, 11·1). The vessels were dissected out while the cord was still immersed in de-oxygenated Krebs saline maintained at a low $O_2$ tension by bubbling with 95% $N_2$/5% $CO_2$. This keeps the gas tensions at fetal levels but allows cooling, which on its own may still lead to contraction through release of prostaglandins (Boura, Boyle & Sinnathuray, 1979). Nevertheless, this method has proved satisfactory in providing viable preparations.

Longitudinal strips of artery (1·0–1·5 cm) and circular strips of vein (0·5 cm lengths of vein were cut along the longitudinal axis) were suspended under 0·3–0·6 g in 50 ml organ baths containing Krebs saline at 37 °C, and the isometric tension was monitored (Grass FTO3c transducers, Grass Model 7 polygraph). The tissues were left to stabilize for 2 h before any experimentation. Neither 5-hydroxytryptamime (5-HT) nor $O_2$ (in the concentration ranges effective on the artery) contracted longitudinal strips of vein ($n = 5$).

The standard mixtures for gassing the saline in two separate groups of preparations were (i) 6% $CO_2$, balance $N_2$, and (ii) 8% $CO_2$, balance $N_2$. Gas tensions in the saline were measured using an Il 213 blood gas analyser. These mixtures produced the following gas tensions respectively: (i) $O_2 = 20 \pm 2$ mmHg, $CO_2 = 30 \pm 1$ mmHg, pH $= 7{\cdot}36 \pm 0{\cdot}02$ and (ii) $O_2 = 20 \pm 2$ mmHg, $CO_2 = 50 \pm 1$ mmHg, pH $= 7{\cdot}28 \pm 0{\cdot}01$ (mean ± S.E. of mean). In a third set of experiments the $NaHCO_3$ concentration of the Krebs solution was reduced to 17 mM. Gas mixture (ii) was used to aerate this. The resulting pH was $7{\cdot}18 \pm 0{\cdot}01$. In all other experiments mixture (ii) was used since the resulting gas tensions and pH are close to that found *in utero* (Pearson, 1976).

Dose–response curves to $O_2$ were constructed non-cumulatively since responses to stepped increments in $O_2$ tension were not always maintained. 5 min exposure to each $O_2$ tension ($P_{O_2}$) was allowed with return to the original mixture for 20 min intervals. The $O_2$ tension was increased with sequential exposures. In each experiment eight different tensions within the range 30–450 mmHg were employed. Response (% of maximum) was plotted against log $P_{O_2}$. In each experiment this relationship was approximately linear. Log $P_{O_2}$ values producing responses of 0, 10, 30, 50, 70, 85 and 100% of the maximum were interpolated from the curve for each tissue: log $P_{O_2}$–response curves were plotted from the means ± S.E. of mean of these. The threshold to contraction (0% of maximum) for each tissue was estimated by extrapolation of its log $P_{O_2}$–response curve to zero response, the assumption being made that the relationship between response and log $P_{O_2}$ remained linear to zero response. The mean $P_{O_2}$ producing 0, 10, 30, 50, 70, 85 and 100% of the maximum was calculated as the antilog of the mean log $P_{O_2}$.

In one series of experiments preparations were incubated with indomethacin (1 or 0·1 $\mu$M) for 30 min, prior to exposure to higher $O_2$ tensions. Control preparations had the vehicle for indomethacin, ethyl alcohol (0·81 or 0·08 mM), added to the organ baths. On completion of the final exposure to raised $O_2$ tension the gas mixture was returned to mixture (ii). After 15 min all the preparations were exposed to 3 $\mu$M-5-HT in order to establish the maximum contractile potential of each tissue. The wet weight of the tissues was then recorded after blotting on filter paper for 1 min.

In other preparations tested with 5-HT, cumulative concentration–response curves were constructed. Responses were expressed as a percentage of the maximum and $pD_2$ values were calculated as $-\log(EC_{50})$, where $EC_{50}$ is the concentration of 5-HT producing a contraction of 50% of the maximum, from interpolation of the log concentration–response curve.

*Drugs*

The drugs used in this study were: 5-hydroxytryptamine creatinine sulphate (5-HT) (Sigma) and indomethacin (Sigma). 5–HT was dissolved in distilled water. Indomethacin was first dissolved in absolute ethyl alcohol and diluted further in distilled water. These drugs were added to the 50 ml organ baths in volumes not greater than 0·5 ml.

*Statistics*

Statistical comparisons of means were made by Student's $t$ test for paired or, where applicable, unpaired data.

## RESULTS

The contraction of human umbilical arterial smooth muscle by $O_2$ was dose dependent (Fig. 1). The threshold to $O_2$ was found to be influenced by pH. The sensitivity to $O_2$ at the different pH values was examined in three separate groups of preparations. At pH 7·36 the threshold was 69 mmHg (mean); at pH 7·28 it was 36 mmHg and at pH 7·18 it was 45 mmHg. The threshold to $O_2$ at pH 7·36 was significantly greater ($P<0·05$) than at pH 7·18 or 7·28. There was no statistically significant difference ($P>0·05$) between the concentration–response curves at pH 7·18 and 7·28. Fig. 1 shows the lower sensitivity to $O_2$ at pH 7·36. pH 7·28 is close to that usually found in the umbilical arterial cord blood.

Maximum contractions were induced at (i) $P_{O_2}$ 282 mmHg (mean), (pH 7·36); (ii) 297 mmHg (pH 7·28) and (iii) 276 mmHg (pH 7·18). There was no significant difference ($P>0·05$) between the mean size of the maximum contractions or the $P_{O_2}$ at which they occurred, at the different pH values. At pH 7·28 the maximum contraction was $0·96 \pm 0·12$ g. At higher $O_2$ tensions the responses were submaximal: at $P_{O_2}$ 460 mmHg (pH 7·28) the contraction induced was $70 \pm 9$% of the maximum.

In preparations incubated with indomethacin (1 or 0·1 $\mu$M) $O_2$ did not induce dose-dependent contractions in individual tissues. This precluded the construction of $P_{O_2}$–response curves as previously described. In this case the contraction induced by $O_2$ (g/g wet tissue weight) was plotted against log $P_{O_2}$ for each tissue and the mean $\pm$ S.E. of mean calculated (Fig. 2). These concentrations of indomethacin (1 or 0·1 $\mu$M) caused neither a relaxation nor contraction of the tissue.

Indomethacin (1 or 0·1 $\mu$M) significantly reduced the contractions at all $P_{O_2}$ values when compared to control preparations which were exposed to the indomethacin vehicle, ethyl alcohol ($P<0·02$). Ethyl alcohol alone did not significantly alter the concentration–response curve to $O_2$ ($P>0·05$) (Fig. 3)

Indomethacin significantly reduced ($P<0·02$) the maximal $O_2$-induced contractions

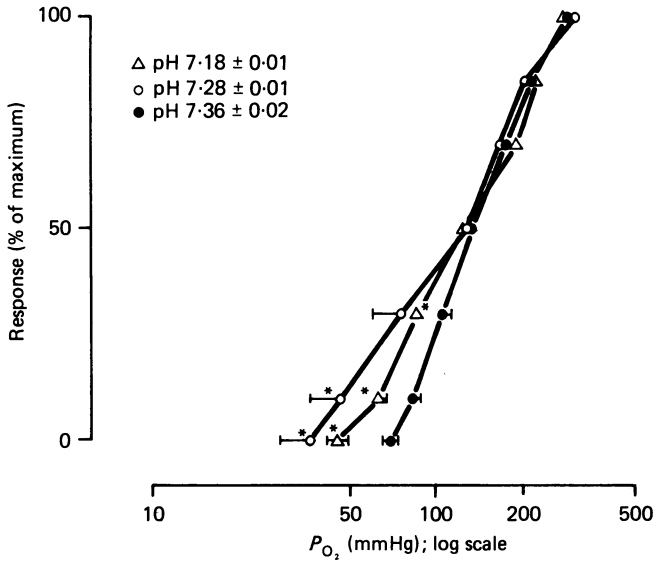


Fig. 1. Concentration–response relationship for contraction of human umbilical artery by oxygen at pH 7·18 (△), pH 7·28 (○) and pH 7·36 (●) ($n = 6$ each group). Asterisks denote significant differences between the curves at pH 7·18 or 7·28 and the curve at pH 7·36. For clarity error bars are shown only at some points. For each individual tissue the response (% of maximum response) was plotted against log $P_{O_2}$. The log $P_{O_2}$ producing 0, 10, 30, 50, 70, 85 and 100 % of the maximum response was interpolated from each curve and the means ± S.E. of means calculated. Log $P_{O_2}$–response curves of the means ± S.E. of means were drawn.

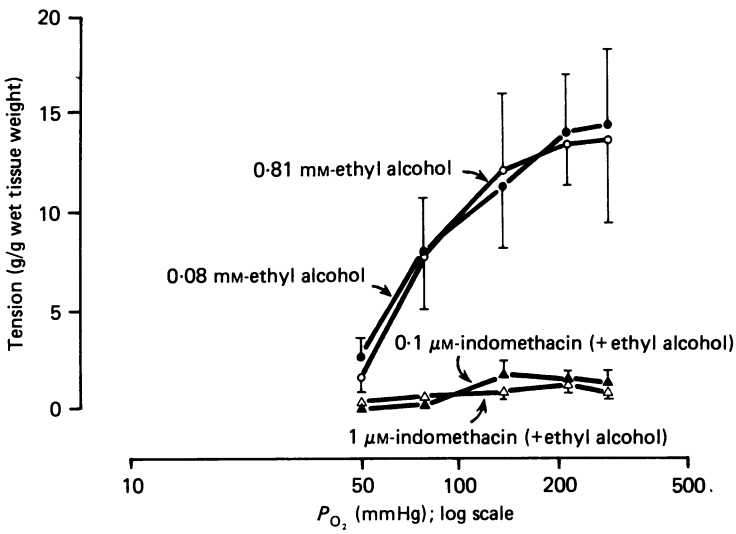


Fig. 2. Indomethacin at 1 μM (△) or 0·1 μM (▲) significantly reduces the $O_2$-induced contractions of human umbilical artery. In paired preparations the vehicle ethyl alcohol at 0·81 mM (○) or 0·08 mM (●) respectively, did not prevent the response to $O_2$ ($n = 6$ each group).

by 72±18% (at 1 μM) and by 87±5% (at 0·1 μM) when compared to control preparations exposed to ethyl alcohol. Ethyl alcohol alone did not significantly change the maximal contractions to $O_2$ or to 5-HT of the preparations ($P>0·05$), which in the controls were 14·9±3·2 g/g tissue weight (mean±S.E. of mean) and 24·4±3·2 g/g tissue weight respectively. There was no statistically significant

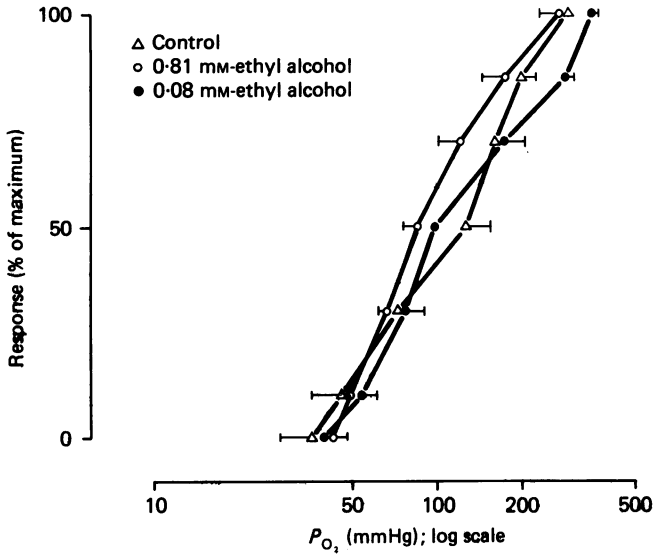


Fig. 3. Ethyl alcohol at 0·81 mM (○) or 0·08 mM (●) did not significantly alter the concentration–response relationship for $O_2$ on human umbilical artery from that already found in separate experiments at pH 7·28 (△) (from Fig. 1) ($n$ = 6 each group).

difference ($P>0·05$) in the mean sizes of the maximal contractions induced by 5-HT between the groups of preparations, whether in the presence of indomethacin (+ ethyl alcohol) or ethyl alcohol alone, when compared to control (non-paired) tissues.

In six venous preparations $O_2$ contracted only one. This contraction was very small (0·03 g) and occurred only when the $O_2$ tension was raised to an unphysiological level (140 mmHg). In paired experiments arteries from the same cords invariably showed concentration-related contractions to stepped increments of $O_2$ tension.

Viability of the venous smooth muscle was tested using 5-HT, a potent agonist in both umbilical artery and vein (McGrath, MacLennan & Stuart-Smith, 1985). 5-HT induced significantly greater contractions in the arterial (0·53±0·1 g) than in the venous preparations (0·25±0·03 g) ($P<0·05$). However, cumulative concentration–response curves to 5-HT were similar. The $pD_2$ for 5-HT in the vein was 7·2±0·2 and in the paired arterial preparations 7·0±0·25 (mean±S.E. of mean).

## DISCUSSION

We have confirmed that the human umbilical artery is contracted by $O_2$. The threshold for this was found to be 36 mmHg at pH 7·28, i.e. at the pH of umbilical blood *in utero* (Pearson 1976). This threshold lies between the normal fetal (15–25 mmHg) and neonatal (100 mmHg) levels and thus potentially represents a major physiological mechanism capable of initiating the change from fetal to neonatal circulations at birth. However, modern obstetric practice of clamping the cord immediately after birth obviates the need for such a mechanism.

The sensitivity to $O_2$ of the smooth muscle at pH 7·18 was not found to be different from that at pH 7·28. This would suggest that the fetal metabolic acidosis which occurs at delivery (Modanlou *et al.* 1973) would not affect the artery's ability to constrict.

We have shown here that prostaglandins are mediators of the $O_2$-induced contraction of the human umbilical artery. This finding is in agreement with the proposal that 'members of the prostaglandin system' are of primary importance in the adjustments of the fetal circulation at birth (pulmonary vasodilation, ductus arteriosus constriction) and that the post-natal rise in blood $O_2$ tension is crucial to these events (Coceani *et al.* 1980).

The vein, however, lacks this $O_2$-induced mechanism. In the case of the preparations of circular muscle this is not due to non-viability since the preparations responded to 5-HT similarly to the arteries. Although the predominant orientation of muscle in the vein is longitudinal (Spivack, 1946) our longitudinal preparations responded to neither 5-HT nor $O_2$. According to our evidence from indomethacin's effect, $O_2$-induced contractions of umbilical artery are mediated by prostaglandins. The umbilical vein is able to synthesize prostaglandins, since Boura *et al.* (1979) found that contraction of the perfused umbilical vein, induced by a reduction in perfusate temperature, was significantly reduced by prior incubation with indomethacin, thus showing that the venous smooth muscle can synthesize constrictor prostaglandins. Therefore $O_2$ does not fail to contract the vein because of an inability of the venous smooth muscle to synthesize constrictor prostaglandins. Presumably the failure is due rather to the lack of the mechanism by which $O_2$ induces prostaglandin synthesis or release.

Since $O_2$ contracts the artery but not the vein, the selective closure of the artery when the $P_{O_2}$ rises after birth could allow the considerable amount of blood contained in the placenta to be transfused to the fetus at birth, a well known observation in the new-born when the cord is not ligated (Windle, 1940). This will not occur if the cord is quickly clamped.

Roach (1972) suggested that the post-natal increase in $O_2$ tension would not be an adequate stimulus to cause vessel closure. However, the change in arterial gas tensions at birth was 'mimicked' in her study by a change in gas mixtures from 10% $O_2$/5% $CO_2$, balance $N_2$, to 20% $O_2$/5% $CO_2$, balance $N_2$. In our experiments this would represent a change in $P_{O_2}$ from 80 to 145 mmHg, at pH 7·36, which does not mimic the physiological changes at birth.

At physiological pH the threshold for $O_2$-induced contraction is not far above the

$O_2$ tension *in utero*, so that fluctuations in $O_2$ tensions or the presence of drugs or physiological agents which sensitize the process (e.g. stimulating constrictor prostaglandin synthesis) could cause vasoconstriction and so reduce fetal blood flow.

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