Annotations

Patent ductus arteriosus: experimental aspects

In the fetus the ductus arteriosus joins the pulmonary trunk to the aorta and is of a calibre similar to these major vessels. Its structure is different since elastic tissue is normally absent from the muscular layers, and its physiological behaviour differs quantitatively and qualitatively from that of the vessels it connects.

In fetal life the ductus provides a bypass to the lungs such that (in the lamb) almost nine-tenths of the right ventricular output is directed to the lower body and placenta.¹ When the lungs expand with gas for the first time, pulmonary arterial pressure falls from slightly above to substantially below aortic level and flow through the ductus reverses.² This, pending constriction and final closure of the ductus, inaugurates a left-to-right shunt recirculating a proportion of left ventricular output through the lungs. As failure of the ductus to close normally is associated with cardiac embarrassment, it is important to understand the mechanisms which promote closure, why they do not normally operate *in utero*, and whether it is possible to assist closure without surgery. Conversely, the adverse postnatal effects of some congenital malformations in which blood can only reach the lungs in any quantity via the ductus are alleviated temporarily by treatment which maintains ductal patency pending corrective surgery. The further possibility exists that substances capable of crossing the placenta can, when administered to the mother, affect the ductus and modify the distribution of fetal cardiac output.

This note is concerned not with ultimate obliteration of the ductus, but with the constriction which takes place shortly after birth.

The fact that initial constriction of the ductus occurs abruptly was first realised nearly 80 years ago, although appreciation of the anatomical idiosyncracies of the fetal circulation is of course much older. Experimental investigation of the mechanisms of ductus closure began about 40 years ago when Barcroft *et al.*³ and Kennedy and Clark⁴ obtained results on guinea-pigs which suggested that oxygen was implicated.

More extensive experiments on fetal lambs showed that increase in O₂ content of the blood consistently constricted the ductus even after destruction of the brain and spinal cord and when

the pulmonary and systemic arterial pressures were stabilised.² The response was also obtained in an isolated heart-ductus-artificial lung preparation and was reversed by anoxia.

In 1963 Kovalčík⁵ confirmed *in vitro* the direct contractile action of oxygen on ductus muscle isolated from guinea-pigs and lambs, and showed that calcium was essential for its occurrence. Since then more penetrating investigation of the oxidative metabolism of ductus muscle has been undertaken⁶⁻⁸ and an extensive range of smooth muscle stimulants investigated. These have included such diverse substances as potassium and acetylcholine which, in appropriate concentration, regularly, or in the case of bradykinin less regularly, constrict ductus muscle.^{5-6,9} They remain useful as experimental tools but seem unlikely participants in natural constriction of the ductus. Since 1972 interest has turned to the part prostaglandins play in the behaviour of this remarkable vessel.

Isolated ductus muscle

Preparations of ductus muscle have been extensively investigated in the form of spiral strips or rings and as the isolated perfused vessel *in vitro* or *in vivo*.

Oxygen. Fay⁶ found that the maximum tension evoked in ductus muscle by an increase of Po₂ was double that produced by acetylcholine or potassium, which stimulate the muscle directly and elicit maintained contraction even under anaerobic conditions. The ductus was equally sensitive to change of luminal or adventitial Po₂, and half-maximal contraction was attained with a Po₂ of 70 mmHg (9.31 kPa) both inside and outside. This makes unlikely the involvement of specialised O₂-sensitive cells. Inhibitors of oxidative phosphorylation reduced the contractile response to O₂ to one-third or less, but had less effect on acetylcholine-induced contractions.

While Kovalčík⁵ had earlier found that the phenothiazines and amyltal prevented the ductus from contracting in response to O₂, in his hands sodium cyanide 1 mmol/l only had a limited effect and he therefore doubted whether the terminal steps of the

electron transport chain were concerned. He suggested a flavoprotein oxidase not inhibited by cyanide might be involved. Under the conditions of his experiments the responses were often slow, and it is possible that cyanide did not remain stable for the length of time required.

Fay and Jöbsis⁷ demonstrated that carbon monoxide also depressed O₂-induced contraction; this effect was reversible by light. Dissociation of the CO-cytochrome a₃ complex preceded muscular contraction by 81 ± 13 (SEM) seconds, thus establishing that the temporal sequence of events is compatible with the concept that O₂ acts via the cytochrome respiratory chain. They found that both O₂ consumption (which was relatively high for smooth muscle) and muscle tension were similarly related to O₂ tension. The O₂ consumption of a ductus partly constricted by O₂ remained unchanged when further tension was elicited by the addition of acetylcholine. This suggested that there is indeed a limitation on cellular respiration imposed by O₂ availability.

Fay⁶ calculated that only when external P_{O₂} exceeded 80 mmHg (10.64 kPa) would all the cells of the ductus smooth muscle be likely to receive sufficient O₂ to sustain their maximum respiratory rate. This would be compatible with a K_m O₂ of about 1 mmHg for the terminal cytochrome a₃. Direct measurement showed very steep oxygen gradients in the muscle.⁸

O₂-evoked contractions of ductus muscle were distinguished by stepwise increases of tension never seen with acetylcholine and other direct stimulants. Fay⁶ likened these to those of other smooth muscles such as taeniae coli which exhibit propagated action potentials, and suggested that these might be fuelled by increased synthesis of high-energy phosphate compounds via the respiratory chain.

Clyman *et al.* found that the maximum tension of O₂-induced contraction of lamb ductus rings increased with increasing gestational age, and also that ductus tissue from immature lambs was very sensitive to light.¹⁰ They thought that this photo-relaxation might account for the relative insensitivity of the immature ductus to stimulation by O₂ *in vitro*. It is interesting that the response of the muscle to direct stimulation with potassium does not vary with age in lambs or guinea-pigs.⁹

Prostaglandins. Clarification of the intricate relationships and actions of these naturally occurring lipid derivatives had proceeded far enough by 1972 for Cocceani and Olley^{11–12} to examine their action on isolated strips of lamb ductus arteriosus. Contrary to expectation, they observed that PGE₁ and PGE₂ relaxed the anaerobic muscle but had less action on

the aerobic (contracted) muscle. The threshold dose was 10^{–9} mol/l; PGA₁ and PGF_{1α} also caused relaxation but only at higher concentrations. Cocceani and Olley¹² suggested that the ductus might be maintained open in fetal life by PGE compounds which also relax the calf ductus. At about the same time, Elliott and Starling¹³ reported that PGF_{2α} contracted calf ductus arteriosus (though at a high dose 10^{–4} mol/l) as Cocceani and Olley¹² had envisaged for this class of compounds in general.

Starling and Elliott¹⁴ gave a systematic account of the responses of the calf ductus to prostaglandins (3.3×10^{-5} mol/l) at O₂ tensions between 15 and 100 mmHg (2.0 and 13.3 kPa). PGF_{2α} was always constrictor and acted synergistically with O₂. PGE₁ caused relaxation at all levels of O₂.

PGE₁ initially caused some contraction followed by relaxation, most pronounced at lower O₂ tensions. They suggested that PGF_{2α} might be responsible for closure of the ductus in calves, and considered that the inhibition of contraction by indomethacin and naproxen implied endogenous synthesis of prostaglandin. In the few experiments attempted, the human ductus behaved in a manner similar to the calf's.

Cocceani *et al.*¹⁵ examined the effects of inhibitors of PGE₂ synthesis on the response of lamb ductus arteriosus to PGE₂ at both low and high O₂ levels. The relaxant effect of PGE₂ was reduced at high O₂ levels, but could be restored after treatment with PG synthetase inhibitors. Possibly the receptiveness of the target site is controlled by the rate of endogenous PG formation. They suggested that at birth PGE formation may increase but the tissue become less sensitive. Olley *et al.*¹⁶ found that ibuprofen (another prostaglandin synthetase inhibitor) caused contraction of the ductus from lambs as young as 90 days' gestation.

Clyman *et al.*¹⁷ did not encounter the insensitivity of well oxygenated lamb ductus muscle to the relaxant action of PGE reported by Starling and Elliott¹⁴ in calf ductus, and suggested on the basis of their own carefully designed investigation that the earlier experiments might have been vitiated by tachyphylaxis. However, Clyman *et al.*¹⁸ routinely used much higher O₂ pressures than Starling and Elliott. Indomethacin-induced contraction of lamb ductus was, as the results of Cocceani *et al.*¹⁵ had indicated, additive to O₂-induced contraction,¹⁸ a situation consistent with endogenous prostaglandin synthesis. In lambs less than 110 days' gestational age indomethacin-induced contraction is greater and the ED₅₀ for relaxation by PGE₂ smaller than in lambs near term.¹⁹

Cocceani *et al.*²⁰ and Clyman *et al.*²¹ have examined the action of several prostaglandins and some of their

derivatives on the lamb ductus. Only $\text{PGF}_{2\alpha}$ and its 15-ketometabolites have been found to cause contraction. Those causing relaxation and effective below 10^{-8} mol/l were PGE_1 , PGE_2 , and their 13, 14 dihydrometabolites. The threshold for PGE_1 is 10^{-12} mol/l and for PGE_2 10^{-11} to 10^{-12} . Both cause contraction above 10^{-6} and 5×10^{-6} mol/l respectively.

The isolated perfused ductus from guinea-pigs and rabbits constricts in response to $\text{PGF}_{2\alpha}$ but dilates with PGE_1 and PGE_2 .²² Dog pup ductus also dilates with PGE_2 .²² However the contractile action of prostaglandin synthetase inhibitors on the guinea-pig ductus is small.²³

Smiesko *et al.*²⁴ found the ductus from fetal guinea-pigs and rabbits maintained in nitrogen constricted when the transmural pressure was suddenly increased. There was a concurrent reduction of sensitivity to vasoconstrictors, and it was suggested that this might be implicated in failure of the ductus to close. Whether the initial myogenic response observed was attributable to prostaglandin release after mechanical stimulation is a matter for speculation.

Prostaglandin synthesis. Homogenates of term ductus of lambs²⁵⁻²⁶ and calves²⁷ converted exogenous arachidonic acid to prostaglandins E_2 , $\text{F}_{2\alpha}$, and 6-keto- $\text{F}_{1\alpha}$. The major product was 6-keto- $\text{F}_{1\alpha}$ which has only weak vasoactive properties, but its short-lived precursor (a prostacyclin) PGI_2 is a weak vasodilator. More PGI_2 was produced by ductus tissue from immature lambs than mature ones.²⁶ Although only about one-tenth of the prostaglandin synthesised is PGE_2 , ductus muscle is so much more sensitive (threshold 10^{-11} mol/l) to it than to PGI_2 (10^{-8} mol/l), that it seems likely to be the major prostaglandin maintaining relaxation of the ductus.

Cultures of vascular endothelium, especially of the umbilical vein, can synthesise PGI_2 and PGE_2 . Angiotensin II 10^{-7} mol/l stimulates production of PGE_2 in such a system.²⁸ Bradykinin has also been reported to increase vascular synthesis of prostaglandins.²⁹ The endothelium of the ductus has not received comparable attention in relation to these compounds.

It is interesting that reduced glutathione which favours PGE_2 synthesis has been found to relax the hypoxic ductus.³⁰

The whole animal

Anatomical investigations. Sharpe and Larsson used whole body freezing techniques³¹ to examine the

effects of prostaglandin administration on the ductus of newborn rats and rabbits. They observed that in newborn rats killed 30 minutes after subcutaneous injection of $\text{PGF}_{2\alpha}$, the ductus diameter was about four times that found in saline-treated controls. The response was dose-dependent over the range 0.2 to 5 $\mu\text{g/g}$ body weight and subsided by 60 minutes after injection. PGE_1 0.2 $\mu\text{g/g}$ caused dilatation comparable with that seen with $\text{PGF}_{2\alpha}$ (1 $\mu\text{g/g}$) and similar results were obtained with both prostaglandins in newborn rabbits. In the absence of hypoxaemia and acidosis it was concluded that both $\text{PGF}_{2\alpha}$ and PGE_1 had delayed ductal closure.

Starling *et al.*³² obtained radiographical evidence in normoxic piglets 3-6 hours of age of dilatation of the ductus in response to infusion of PGE_1 or PGE_2 (1-4 $\mu\text{g/kg}$ per min) or PGA_1 or PGA_2 (20-40 $\mu\text{g/kg}$ per min) into the aortic arch; the left to right shunt appeared larger.

The obstetric use of the prostaglandin synthetase inhibitors, indomethacin and acetylsalicylic acid, in conjunction with the fact that the concentration of indomethacin in human fetal blood can reach 1.6 times that in the mother³³ prompted Sharpe *et al.*³⁴⁻³⁵ to investigate the fetal consequences of maternal administration of indomethacin 15 mg/kg. This caused constriction of the ductus in near term fetal rabbits and rats. Rat pups so exposed *in utero* were cyanosed and lethargic on delivery with distressed breathing. Doses of indomethacin (2.5 mg/kg) in the clinical range and acetylsalicylic acid (50-100 mg/kg) were also effective.

Indomethacin 2×5 mg/kg per day was given orally to 10 near term ewes for 3 days with the final dose 1-3 hours before caesarean section.³⁶ The lambs whose blood-gases were in the normal range were delivered into an hypoxic atmosphere (<14 mmHg 1.9 kPa), the cord was tied and the chest opened before immersion in liquid nitrogen. The cross-sectional areas of the ductus ranged from 0.84-11.03 mm² and were less ($P < 0.05$) than controls (range 9.16-53.23 mm²).

Physiological experiments. Chronic ligation of the ductus in fetal lambs has been reported compatible with live birth³⁷ although this is not the case with fetal dogs³⁸ which survived *in utero* only until shortly before parturition. When the ductus was tied in 4 fetal lambs, the average pressure difference between pulmonary trunk and aorta under maternal anaesthesia rose from 1.2 to 20.6 mmHg (0.16 to 2.7 kPa).³⁷ These lambs survived for 9-36 days, they had 34% more ventricular muscle than littermates, and changes in the media of small muscular pulmonary arteries were found.

Administration of acetylsalicylic acid 55-90

mg/kg estimated body weight to catheterised fetal lambs *in utero* increased the pressure drop from pulmonary artery to aorta from 1.5 to 11.2 mmHg (0.20 to 1.49 kPa), and decreased the fraction of right ventricular output traversing the ductus from 88.5 to 69.7%. The absolute decrease averaged 15.9%.¹ In two lambs with pulmonary hypertension, IV infusion of PGE₁ 0.1 mg/kg per minute restored normal levels of pulmonary arterial pressure. When the infusion was stopped pulmonary pressure rose again.¹ Substantial changes in the distribution of cardiac output (measured with radioactive microspheres) were found during administration of acetylsalicylic acid. For example, that to the liver was decreased by 40% and that to the lungs increased by 196%.¹ Though blood-gas tensions were unaffected, if such large changes in organ flow had persisted with continuous treatment they might have had important consequences. Indomethacin (0.005 mg/kg) not only caused profound ductus constriction in fetal lambs but also reduced circulating levels of PGE.³⁹ Infusion of PGE reversed the constriction but did not raise circulating levels. Slow infusion of indomethacin (1 mg/kg maternal bodyweight) into pregnant ewes raised the pressure gradient across the fetal ductus from 2.7 ± 2.0 to 14.1 ± 5.9 mmHg during the next 1–6 hours. The increase began within 30 minutes, was not attributable to a fall of systemic pressure, but was reduced by infusion of PGE₁ in two lambs. Nine lambs which had not received PGE₁ were found dead the next day.⁴⁰

Thibeault *et al.*⁴¹ found that in fetal lambs treated with hydrocortisone for several days, the ductus was functionally closed or constricted compared with controls. Corticosteroids can inhibit prostaglandin release, so that this observation would be consistent with a role for PGE in the maintenance of ductus patency.

Circulating prostaglandins. PGE levels are high (10^{-9} mol/l) in plasma obtained from catheterised lambs *in utero* especially towards term.⁴² Levels fall in the first few days of life, as in human infants where they appear unaffected by gestational age at birth.⁴³

Discussion

Almost all *in vitro* work has used an anoxic (<14 mmHg) relaxed ductus for the investigation of contractile agents, and one maximally constricted in an O₂ environment of several hundred mmHg for detection of relaxant action. Whether the apparent difference of sensitivity to O₂ of guinea-pig and lamb isolated ductus is solely due to the larger size of, and greater dependence on, vasa vasorum of the latter is

perhaps unlikely, since the even larger ductus of the calf is contracted at a Po₂ of 100 mmHg.

Clarification of such discrepancies may well require more detailed anatomical knowledge and discrimination between the synthetic abilities of the various tissues comprising the ductus in various species. However no doubt exists as to the graded response of the ductus to increasing O₂ tension both *in vitro*^{6,9} and *in vivo*.^{2,44} The stimulation of increased O₂ tension remains unchallenged as a trigger for post- (and pre-) natal constriction of the ductus. Although synthesis of prostaglandins requires oxygen,⁴⁵ it appears possible at the rather low O₂ tensions in the fetus.

Prostaglandins in plasma may participate in the control of vascular tone *in vivo* in addition to that produced by endogenous synthesis within the vessel wall itself. In general, the behaviour *in vitro* of isolated ductus tissue to exogenous prostaglandins and to prostaglandin synthetase inhibitors accounts for the observations *in vivo*. The undoubted presence of autonomic innervation of the ductus⁴⁶ has not been shown to play an important part in its behaviour.

The possible roles of bradykinin²⁹ and angiotensin II²⁸ in PGE synthesis may deserve closer examination. However, if in the ductus, as appears both PGE and PGF_{2α} can be produced, administration of a prostaglandin synthetase inhibitor might predominantly promote constriction by inhibition of PGE production, or dilatation by inhibition of PGF_{2α} production. In addition, sheep blood contains an enzyme, with a pH optimum about 7, capable of reducing PGE₂ to PGF_{2α}.⁴⁷ Thus the final outcome may not be easily predictable in the whole organism.

The part played by prostaglandins in relation to the contractile action of O₂ on the ductus, which is believed to be mediated by an increased rate of synthesis of high-energy phosphate compounds,^{6–8} is not understood. Prostaglandins are lipid-soluble and their involvement in control of intracellular Ca⁺⁺ at the level of the mitochondrial membrane in other systems suggests they could be responsible,⁴⁸ though further sites of action such as the sarcomere membrane must remain possibilities.

Whole animal.

Fetus. If the ductus is totally (by ligation), or partly (by administration of prostaglandin synthetase inhibitors), prevented from performing its normal fetal function there is substantial evidence that pathological consequences can follow.^{34–35 37–38} Any increase of pulmonary arterial pressure could be mitigated by increased flow through the foramen

ovale.³⁸ However, while brief administration of prostaglandin synthetase inhibitors may have only a short-lived effect on PGE synthesis, in ductus muscle the consequences of prolonged inhibition of prostaglandin synthesis there and elsewhere cannot be discounted. Nor can it be assumed, in the case of compounds which cross the placenta, that maternal plasma concentrations of the inhibitor are any indication of those in the fetus.^{33 49}

Newborn A histological study of human patent ducts shows that all those from subjects over age 4 months have abnormal subintimal elastic tissue.⁵⁰ This anomaly was also present at all ages from birth to 4 months together with histologically normal ducts at various stages of closure. It is therefore possible that congenital abnormality precludes both spontaneous or pharmacologically-assisted closure of some ducts.

In anatomically normal newborns, which are not hypoxaemic, failure of the ductus to close could be attributable to endogenous synthesis of PGE rather than PGF, but there is no direct evidence on this point. Doubt has been expressed about whether concentrations of PGF_{2α} sufficiently high to cause ductus constriction are likely to occur naturally. As far as plasma levels are concerned, both PGE and PGF were raised in 6 of 7 human infants with patent ductus arteriosus (PDA).⁵¹ In two cases a relative increase of plasma PGE with respect to PGF coincided with cardiac failure due to a large left-to-right shunt through a patent ductus.⁵²

Administration of prostaglandin synthetase inhibitors to promote closure of the ductus cannot avoid effects on other organs. A well documented example is the inhibition of renal blood flow and urine flow in newborn lambs.⁵³ Elimination of indomethacin by premature infants⁵⁴ is as slow as that in the fetus.³³

In infants in whom the pulmonary blood flow is largely dependent on a PDA and presumably on adequate endogenous synthesis of PGE, O₂ levels, though low, may well exceed fetal levels and perhaps hinder any dilator effect of PGE whether endogenous or exogenous.

The histology of the ductus in cases of ductus-dependent cardiac anomaly, treated with PGE, suggested structural weakening due to intimal lacerations, interruption of internal elastic laminae, and medial oedema.⁵⁵ Plasma levels of PGE were unaffected by ductus ligation.⁵¹

In the newborn as in the adult⁵⁶⁻⁵⁷ prostaglandins are metabolised in the lungs. The much larger pulmonary blood flow of the newborn can be expected to dispose of any surplus prostaglandins more readily than the fetus.

Conclusions.

- (1) The Po₂ of fetal blood is insufficient to constrict the ductus arteriosus in the face of its endogenous synthesis of prostaglandin E.
- (2) Closure of the ductus at birth is initiated by the increased Po₂ of circulating blood as pulmonary ventilation is established. The participation of PGF_{2α} is improbable as relatively large concentrations are required to constrict ductus muscle and in some experiments a dilator action was observed.
- (3) Administration of PGE has been shown to dilate the partly constricted postnatal ductus *in vivo*.
- (4) Some inhibitors of prostaglandin synthetase cross the placenta; these may attain a concentration in fetal plasma exceeding that in the mother, and have been shown experimentally to promote constriction or closure of the ductus *in utero*. The haemodynamic consequences of such closure include changes in the pulmonary vessels which may preclude the normal development of vascular conductance after birth.
- (5) If the ductus remains open postnatally at reasonable levels of oxygenation, prostaglandin synthetase inhibitors may assist closure as it seems unlikely that any constrictor effects of PGF_{2α} would dominate the situation. However, the multitude of other prostaglandin synthetase systems in the body would also be more or less inhibited. The possibility of adverse consequences of prolonged treatment requires detailed analysis.

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