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. 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. 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 idiosyncrasies 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. and Kennedy and Clark 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. The response was also obtained in an isolated heart-ductus-artificial lung preparation and was reversed by anoxia.

In 1963 Kovalčík 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 undertaken 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.

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. Fay 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 Kovalčík had earlier found that the phenothiazines and amytyl 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 Jöbsis demonstrated that carbon monoxide also depressed O$_2$-induced contraction; this effect was reversible by light. Dissociation of the CO-cytochrome a$_3$ complex preceded muscular contraction by 81 ± 13 (SEM) seconds, thus establishing that the temporal sequence of events is compatible with the concept that O$_2$ acts via the cytochrome respiratory chain. They found that both O$_2$ consumption (which was relatively high for smooth muscle) and muscle tension were similarly related to O$_2$ tension. The O$_2$ consumption of a ductus partly constricted by O$_2$ 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 O$_2$ availability.

Fay calculated that only when external PO$_2$ exceeded 80 mmHg (10.64 kPa) would all the cells of the ductus smooth muscle be likely to receive sufficient O$_2$ to sustain their maximum respiratory rate. This would be compatible with a K$_m$ O$_2$ of about 1 mmHg for the terminal cytochrome a$_3$. Direct measurement showed very steep oxygen gradients in the muscle.

O$_2$-evoked contractions of ductus muscle were distinguished by stepwise increases of tension never seen with acetylcholine and other direct stimulants. Fay 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 O$_2$-induced contraction of lamb ductus rings increased with increasing gestational age, and also that ductus tissue from immature lambs was very sensitive to light. They thought that this photo-relaxation might account for the relative insensitivity of the immature ductus to stimulation by O$_2$ 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.

**Prostaglandins.** Clarification of the intricate relationships and actions of these naturally occurring lipid derivatives had proceeded far enough by 1972 for Coceani and Olley to examine their action on isolated strips of lamb ductus arteriosus. Contrary to expectation, they observed that PGE$_1$ and PGE$_2$ relaxed the anaerobic muscle but had less action on the aerobic (contracted) muscle. The threshold dose was 10$^{-9}$ mol/l; PGA$_1$ and PGF$_2\alpha$ also caused relaxation but only at higher concentrations. Coceani and Olley 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 Starling reported that PGF$_2\alpha$ contracted calf ductus arteriosus (though at a high dose 10$^{-4}$ mol/l) as Coceani and Olley had envisaged for this class of compounds in general. Starling and Elliott gave a systematic account of the responses of the calf ductus to prostaglandins (3·3 × 10$^{-5}$ mol/l) at O$_2$ tensions between 15 and 100 mmHg (2·0 and 13·3 kPa). PGF$_2\alpha$ was always constrictor and acted synergistically with O$_2$. PGE$_1$ caused relaxation at all levels of O$_2$.

PGE$_2$ initially caused some contraction followed by relaxation, most pronounced at lower O$_2$ tensions. They suggested that PGF$_2\alpha$ 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.

Coceani et al. examined the effects of inhibitors of PGE$_2$ synthesis on the response of lamb ductus arteriosus to PGE$_2$ at both low and high O$_2$ levels. The relaxant effect of PGE$_2$ was reduced at high O$_2$ 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. found that ibuprofen (another prostaglandin synthetase inhibitor) caused contraction of the ductus from lambs as young as 90 days' gestation.

Clyman et al. did not encounter the insensitivity of well oxygenated lamb ductus muscle to the relaxant action of PGE reported by Starling and Elliott 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. routinely used much higher O$_2$ pressures than Starling and Elliott. Indomethacin-induced contraction of lamb ductus was, as the results of Coceani et al. had indicated, additive to O$_2$-induced contraction, a situation consistent with endogenous prostaglandin synthesis. In lambs less than 110 days' gestational age indomethacin-induced contraction is greater and the ED$_{50}$ for relaxation by PGE$_2$ smaller than in lambs near term.

Coceani et al. and Clyman et al. have examined the action of several prostaglandins and some of their
derivatives on the lamb ductus. Only PGF\textsubscript{2\alpha} and its 
15-ketometabolites have been found to cause contraction. Those causing relaxation and effective 
below $10^{-8}$ mol/l were PGE\textsubscript{1}, PGE\textsubscript{2}, and their 13, 14 
dihydrometabolites. The threshold for PGE\textsubscript{1} is 
$10^{-12}$ mol/l and for PGE\textsubscript{2} $10^{-11}$ to $10^{-12}$. Both cause 
contraction above $10^{-6}$ and $5 \times 10^{-6}$ mol/l respectively.

The isolated perfused ductus from guinea-pigs and 
rabbits constricts in response to PGF\textsubscript{2\alpha} but dilates with 
PGE\textsubscript{1} and PGE\textsubscript{2}.\textsuperscript{29} Dog pup ductus also dilates 
with PGE.\textsuperscript{29} However the contractile action of 
prostaglandin synthetase inhibitors on the guinea-pig 
ductus is small.\textsuperscript{33}

Smiesko et al.\textsuperscript{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 lambs\textsuperscript{25–26} and calves\textsuperscript{27} converted exogenous 
arachidonic acid to prostaglandins E\textsubscript{2}, F\textsubscript{2\alpha}, and 
6-keto-F\textsubscript{1\alpha}. The major product was 6-keto-F\textsubscript{1\alpha} 
which has only weak vasoactive properties, but its 
short-lived precursor (a prostacyclin) PGI\textsubscript{2} is a weak 
vasodilator. More PGI\textsubscript{2} was produced by ductus 
tissue from immature lambs than mature ones.\textsuperscript{28} 
Although only about one-tenth of the prostaglandin 
synthesised is PGE\textsubscript{2}, ductus muscle is so much 
more sensitive (threshold $10^{-11}$ mol/l) to it than to 
PGI\textsubscript{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\textsubscript{2} and PGE. 
Angiotensin II $10^{-7}$ mol/l stimulates production of 
PGE in such a system.\textsuperscript{28} Bradykinin has also been 
reported to increase vascular synthesis of 
prostaglandins.\textsuperscript{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 synthesis has been found to relax the 
hypoxic ductus.\textsuperscript{30}

**The whole animal**

**Anatomical investigations.** Sharpe and Larsson used 
whole body freezing techniques\textsuperscript{31} 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 PGF\textsubscript{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 μg/g body weight and subsided by 60 minutes after 
injection. PGE, 0.2 μg/g caused dilatation comparable 
with that seen with PGF\textsubscript{2\alpha} (1 μg/g) and similar 
results were obtained with both prostaglandins in 
newborn rabbits. In the absence of hypoxaemia and 
acidosis it was concluded that both PGF\textsubscript{2\alpha} and 
PGE\textsubscript{1} had delayed ductal closure.

Starling et al.\textsuperscript{30} obtained radiographical evidence 
in normoxic piglets 3–6 hours of age of dilatation of 
the ductus in response to infusion of PGE\textsubscript{1} or PGE\textsubscript{2} 
(1–4 μg/kg per min) or PGA\textsubscript{1} or PGA\textsubscript{2} (20–40 μg/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 mother\textsuperscript{32} prompted Sharpe et al.\textsuperscript{34–36} 
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 
cyanozed 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.\textsuperscript{30} 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 
mm\textsuperscript{2} and were less (P < 0·05) than controls (range 
9·16–53·23 mm\textsuperscript{2}).

**Physiological experiments.** Chronic ligation of the 
ductus in fetal lambs has been reported compatible 
with live birth\textsuperscript{37} although this is not the case with 
fetal dogs\textsuperscript{38} 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).\textsuperscript{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.38 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 (10–4 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 vitro6 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 ductus48 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 PGF2α can be produced, administration of a prostaglandin synthetase inhibitor might predominantly promote constriction by inhibition of PGE production, or dilatation by inhibition of PGF2α production. In addition, sheep blood contains an enzyme, with a pH optimum about 7, capable of reducing PGE2 to PGF2α.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,4–8 is not understood. Prostaglandins are lipid-soluble and their involvement in control of intracellular Ca2+ 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.34–36 37–38 Any increase of pulmonary arterial pressure could be mitigated by increased flow through the foramen
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.\(^{49}\)

**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\(_{2\alpha}\) 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 duct.\(^{52}\)

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 infants\(^{54}\) 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, O\(_2\) 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 adult 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 PO\(_2\) 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 PO\(_2\) of circulating blood as pulmonary ventilation is established. The participation of PGF\(_{2\alpha}\) 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 vivo. 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\(_{2\alpha}\) 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.

**References**

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