Responses of the post-term arterial duct to oxygen, prostaglandin E_2, and the nitric oxide donor, 3-morpholinosydnonimine, in lambs and their clinical implications

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Abstract

***Background*—Nitric oxide is a potent dilator of the pulmonary vasculature. There have been no previous reports on the action of nitric oxide on the arterial duct.**

***Objectives*—To determine the responses of isolated post-term arterial duct rings from lambs to oxygen, prostaglandin E_2 (PGE_2) and the nitric oxide donor, 3-morpholinosydnonimine (SIN-1).**

***Setting*—Experimental laboratory.**

***Subjects*—Six neonatal lambs.**

***Methods*—Lambs aged 1-5 days were killed and the arterial duct and aorta excised and cut into rings. These were mounted on tension gauges in organ baths containing Krebs-Henseleit solution. Rings were exposed to increasing concentrations of oxygen, PGE_2 and after preconstriction with potassium (40 mmol/l) to SIN-1. Tension and relaxation responses were recorded.**

***Results*—Increased oxygen tension resulted in increased tension in the ductal rings above 88·9 mm Hg as previously described. No response to PGE_2 occurred before or after ductal rings were exposed to oxygen. SIN-1 caused relaxation of smooth muscle in the arterial duct to a similar degree as that in the aortic rings.**

***Conclusions*—As previously shown, oxygen is a potent constrictor of the arterial duct. The post-term arterial duct does not relax in response to PGE_2 possibly as a result of inactivation by oxygen of the special sensitivity of the duct to PGE_2. SIN-1 is a potent smooth muscle relaxing agent in the term arterial duct and may have a role in the initial management of neonates with duct dependent pulmonary circulation.**

(*Br Heart J* 1995;73:177–181)

Keywords: arterial duct, oxygen, prostaglandins, nitric oxide.

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Accepted for publication 9 August 1994

Closure of the arterial duct shortly after birth is a physiological process that occurs as a result of smooth muscle constriction within the duct.[1-3] The mechanism of closure is not known but is thought to be mediated through a cytochrome P_{450} leading to endothelin release which constricts the arterial duct. Oxygen and other factors may cause conformational changes in the cytochrome P_{450} that initiate the process.[4-6] Ductal patency is maintained by prostaglandins, mainly PGE_2, before birth.[7] This group of drugs has found a significant clinical role in the management of neonates with congenital heart lesions with duct dependent circulations, in whom physiological closure of the arterial duct is potentially fatal.[8]

Clinical experience has shown that prostaglandins have a variable effect on ductal patency, resulting in inadequate dilatation on occasion.[8] This is probably not related to gestational age although Starling and Elliott[9] reported increasing sensitivity of the arterial duct to certain prostaglandins in laboratory experiments with increasing gestational age. The majority of neonates receiving prostaglandin infusions are born at term. An agent that causes both pulmonary vasodilatation and ductal dilatation might have a significant role to play in the management of neonates with duct dependent pulmonary circulations such as tricuspid atresia, pulmonary atresia, or tetralogy of Fallot.

The endothelium derived relaxing factor, nitric oxide, has been used recently in both animal models[10] and clinical situations[11] to relax the pulmonary vasculature and hence reduce pulmonary vascular resistance. There have been no previous reports on the action of nitric oxide on the arterial duct. After birth the arterial duct is usually undergoing a process of degeneration and the intimal layer is abnormal. Intimal cushions and postnatal intimal proliferation are present. The endothelium may be absent in places.[12] It is likely that endothelial function is abnormal and therefore the use of acetylcholine to induce nitric oxide release would be inappropriate. This study examines the term arterial duct response in lambs to the nitric oxide donor 3-morpholinosydnonimine (SIN-1).

Materials and methods

The investigation was performed in accordance with the Home Office Guidance on the operation of the Animals (Scientific Procedures) Act 1986, published by Her Majesty's Stationery Office, London.

We studied six Suffolk and Cambridge cross lambs. They were delivered at term, without artificial aids, (145 days gestation) and killed 12 h-5 days after birth. Their mean (range) weight was 4·2 (3·5–5·0) kg. Using

either halothane/oxygen or pentobarbitone the lambs were anaesthetised and the chest opened via a left thoracotomy. Exsanguination was performed by incising the cardiac apex. The arterial duct was removed, along with a length of aorta and pulmonary artery, rinsed of blood and placed in Krebs-Henseleit solution containing (mmol/l) sodium chloride 118, potassium chloride 4·7, sodium bicarbonate 25, potassium dihydrogen phosphate 1, magnesium sulphate 0·9, calcium chloride 2·5, glucose 11·1 at 4°C.

Excised ducts were cut into two to three rings (1–2 mm wide) and mounted within water-jacketed organ baths containing Krebs-Henseleit solution (30 ml) at 37°C between stainless steel rods (bent 21 gauge needles) and 00 gauge silk thread attached to a tension transducer (Dynamometer UF1 Tension Transducer). Tension transducers were connected to Lectromed Multitrace 2 amplifiers and recorders. These were calibrated to measure tension up to 2 g across the scale and were used to measure force produced by the rings in response to added agents. The initial gas mixture bubbled through the baths was 95% nitrogen and 5% carbon dioxide. Alongside the ductal rings, in a second organ bath, a ring of aorta was mounted to serve as a control and to confirm tissue viability. Aortic rings were generally about one third larger than the ductal rings. The gas mixture in the aortic organ bath was 95% oxygen and 5% carbon dioxide.

In all cases the excised duct was constricted, but obliteration of the lumen had not taken place. Constriction was complete in five lambs but in the youngest a small lumen remained.

Tension of about 1 g was applied to the ductal rings after mounting and they were left to equilibrate for 1–2 h. Increasing concentrations of PGE_2 were added to the organ baths after equilibration and gas analysis. The gas mixtures were changed after washing out of the prostaglandin by switching to output from other cylinders and ductal constriction in response to increasing oxygen tension was recorded. Oxygen tension (Po_2) was measured by withdrawing 1 ml of Krebs-Henseleit solution from the organ bath and injecting it into a Corning 158 pH/blood gas analyser (Corning, Halstead, Essex). Cylinder 1 contained 95% nitrogen, 5% carbon dioxide, and no oxygen (Krebs-Henseleit Po_2 = 22·6-56·2 mm Hg); cylinder 2, 2·5% oxygen, 92·5% nitrogen, and 5% carbon dioxide (Po_2 = 38·5–74·2 mm Hg); cylinder 3, 15% oxygen, 80% nitrogen, and 5% carbon dioxide (Po_2 = 88·9–144·3 mm Hg); cylinder 4, 30% oxygen, 65% nitrogen, and 5% carbon dioxide (Po_2 = 125·0–248·7 mm Hg); cylinder 5, 95% oxygen, no nitrogen, and 5% carbon dioxide (Po_2 = 477·0–779·0 mm Hg). PGE_2 was again added in increasing concentrations with 95% oxygen, 5% carbon dioxide being bubbled through the organ bath.

Subsequent experiments were performed with the 95% oxygen and 5% carbon dioxide mixture after washing out of the prostaglandin. Stepwise aliquots of a solution of 1·0 mol/l potassium chloride were added to the organ bath to increase the potassium concentration from 10 to 60 mmol/l and obtain a dose response curve.

Submaximally preconstriction of the arterial duct rings was achieved with a potassium concentration of 40 mmol/l. Acetylcholine (10^{-7}-10^{-4} mol/l) was then added to the arterial duct baths. Drugs were washed out and potassium (40 mmol/l) added followed by increasing concentrations of SIN-1 (10^{-7}-10^{-4} mmol/l). Aortic rings were exposed to prostaglandin, potassium and SIN-1. Contraction and percentage relaxation of the precontracted level were measured and recorded.

DRUGS

Drugs used and their sources were as follows: gas mixtures (BOC, Special Gases, Guildford, Surrey); PGE_2 (gift of Upjohn, West Sussex); acetylcholine (Sigma, Poole, Dorset); and SIN-1 (gift of Cassella-Reidel Pharma, Frankfurt). Acetylcholine and SIN-1 were dissolved in distilled water and frozen in aliquots, with a fresh aliquot thawed each day. Fresh prostaglandin ampoules were used daily. All drugs were diluted in Krebs-Henseleit solution to make up the range of concentrations.

STATISTICAL ANALYSIS

Contraction responses are expressed as mean (SD) gram increase in isometric tension. Percentage relaxation of the precontracted level were calculated as mean (SD) percentage of the preconstricted tension. Data were compared by unpaired t tests. Differences of $p \leqslant 0{\cdot}05$ were regarded as significant.

Results

The post-term duct did not constrict markedly under anaerobic conditions during equilibration but rapidly achieved a steady state with loss of between 0·05 and 0·2 g tension over 3–5 min probably related to straightening of the silk thread. Aortic rings developed between 0·4 and 0·8 g of resting tension over 10–30 min.

RESPONSES TO PGE_2

PGE_2, when given in concentrations of 10^{-7}-10^{-5} mmol/l had no effect on the arterial duct under either anaerobic or aerobic conditions. Transient constriction (lasting 3 min) of 0·16–0·21 g occurred in three rings with 10^{-4} mmol/l prostaglandin. The control aortic ring in aerobic conditions did not respond.

RESPONSES TO OXYGEN

Whilst the ability of the ductal rings to relax in response to PGE_2 appears to be lost post-term, all the ducts constricted when the oxygen concentration was 88·9–144·3 mm Hg and higher, with only small variations between ducts and between oxygen concentrations (fig 1). Responses occurred within 1–2 min.

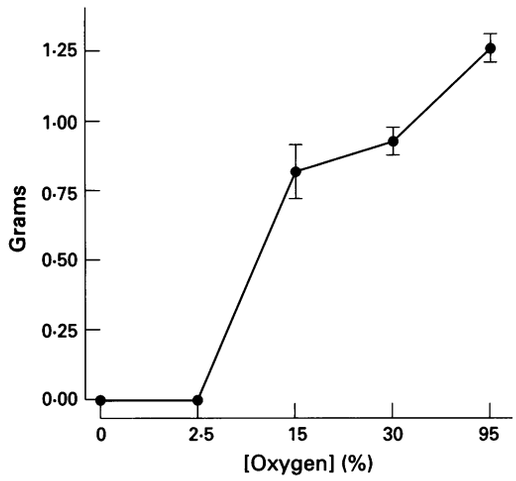

Figure 1 Response of the arterial duct rings to increasing oxygen tension. The x axis represents the percentage of oxygen in each gas cylinder. (See the text for the equivalent oxygen tensions in the organ baths.)

RESPONSES TO POTASSIUM

Both arterial ducts and aortas constricted with potassium (fig 2(A) and (B)). The tension produced by the duct rings was about equal to that of the aortic rings (P = NS, unpaired *t* test).

RESPONSES TO SIN-1

Figure 3 shows an example of a trace from one of the arterial duct rings. Figure 4(A) and (B) shows the response of the ductal and aortic rings to the nitric oxide donor, SIN-1. Ductal relaxation with SIN-1 after constriction is profound and maximal at concentrations of 10^{-7}-10^{-5} mol/l. Aortic rings taken at the same time from the same animals relaxed with SIN-1 to a similar degree (P = NS, unpaired *t* test).

There was no difference in response related to increasing postnatal age. The ductal rings did not relax in response to acetylcholine. Histological examination of a representative number of the ductal rings showed the normal appearance of intimal cushions and that the endothelium was ragged or missing.

Discussion

Our results in isolated tissue experiments show that the nitric oxide donor SIN-1 relaxes preconstricted post-term arterial duct rings in lambs. The arterial duct rings do not relax to PGE_2 under anaerobic or aerobic conditions. Oxygen is a potent vasoconstrictor of the post-term arterial duct in lambs.

In contrast to the findings of Coceani and Olley[7] who removed the arterial duct from fetal lambs, the post-term duct did not constrict markedly under anaerobic conditions during equilibration but rapidly achieved a steady state. The transient slight constriction of the ductal rings at higher concentrations is probably a direct receptor effect. Both Coceani and Olley[7] and Starling and Elliot[9] have shown that the arterial duct in fetal lambs relaxes in response to prostaglandins under anaerobic conditions and that the effect is markedly attenuated under aerobic conditions. Clyman *et al*[13] showed that prostaglandin E_1 was effective in relaxing the arterial duct in vitro under high oxygen concentrations, but they could only demonstrate the effect in the short term. In our experiments all the lambs had been breathing air for at least 12 h, during which time the ducts were exposed to physiological concentrations of oxygen and had undergone normal postnatal constriction in contrast to the experimental animals of the earlier investigators and the hypoxic human neonates with duct dependent circulation. In vitro pharmacological preparations are not, in general, accurate models of the in vivo situation. In respect to prostaglandin, however, the in vitro effect in arterial ducts in fetal lambs[7] and the in vivo effect of prostaglandin infusion in neonates[14] was successfully shown to be similar. We suggest, from our data, that chronic exposure to oxygen may switch off the sensitivity of the duct to prostaglandins. Evidence to support this view is the relative insensitivity to prostaglandin of neonates with aortic coarctation.[8] Olley and Coceani,[7] Clyman *et al*[13] and Kovalcik[15] all demonstrated that oxygen, over the short term, attenuates the effect of

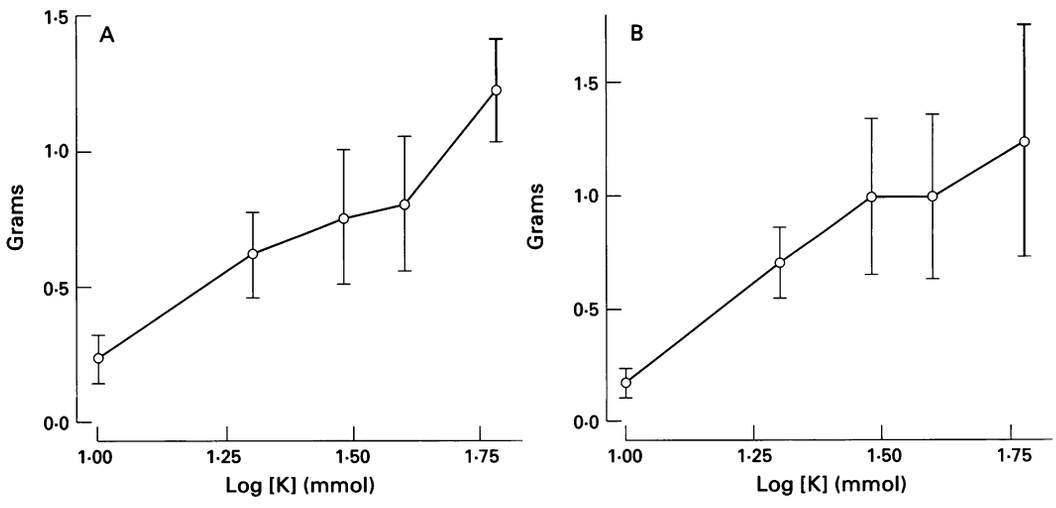

Figure 2 Responses of (A) arterial duct rings and (B) aortic rings to increasing concentrations of potassium. The oxygen constricted duct, without potassium, was considered as the steady state. (The x axis is calibrated logarithmically but in the experiments the potassium dose was increased arithmetically.)

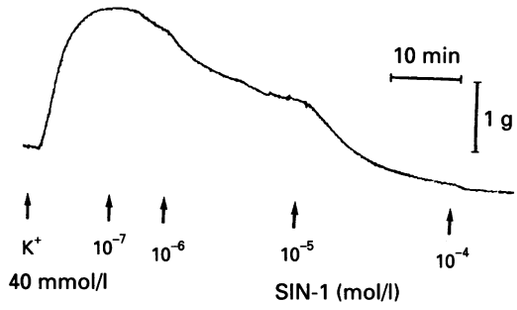

Figure 3 Trace of force against time from one of the arterial duct rings. Initial exposure to potassium 40 mmol/l leads to constriction and is followed by relaxation in response to increasing concentrations of SIN-1. The arrows indicate time of addition of agents.

prostaglandin. The "switch", as yet undefined, may be a physiological protective mechanism that ensures the arterial duct remains closed after birth. This mechanism works alongside the positive constrictive effects of oxygen. Further work is required to clarify this point.

No relaxation response to acetylcholine was observed. Stimulation of nitric oxide release by acetylcholine from the endothelium might be expected not to occur as the intima and endothelium are undergoing a degenerative process at term[16] and are unlikely to be functionally normal. Histological examination of the ductal rings confirmed that the endothelium was ragged or absent from the rings.

Since the first descriptions of endothelial derived relaxing factor[17] and its subsequent identification as nitric oxide[18] the sites in which it has been found and its uses have multiplied. However, there have been no previous reports on its effects on the arterial duct. Our results show that the profound effect of the nitric oxide donor, SIN-1, on the duct was similar to that on the aorta. The relaxation induced by SIN-1 was greater than 100%, suggesting that not only was the constriction induced by potassium overcome, but also some of basal tone induced by oxygen. This is of some significance if nitric oxide is to be used in the clinical situation to overcome the constricting effects of oxygen.

LIMITATIONS OF THE STUDY AND FUTURE WORK

It may have been more physiologically appropriate to use oxygen tensions in the in vivo range while studying arterial duct responses to SIN-1; however, perfusion of the tissue rings in the organ baths is not physiological. Although oxygen tension in the organ baths was very high when using the 95% oxygen cylinder, it may reflect only availability and exposure of the gas to the tissue on the surface of the ring.

We used the nitric oxide donor SIN-1 in place of nitric oxide gas for logistic reasons and because we considered that the effects could be compared. Nitric oxide in the clinical situation is obtained from a gas cylinder via a ventilation circuit or head-box. The advantage of administration via the lungs is the avoidance of any systemic effect because of rapid metabolism.

Conclusion

Inhaled nitric oxide has found an increasing role in the management of complex congenital heart disease where there is high or fluctuant pulmonary artery pressure.[11] Our experimental data suggest that nitric oxide may also be useful in the early management of neonates with duct dependent pulmonary circulation (for example, tricuspid or pulmonary atresia) dilating not only the pulmonary vasculature but also the arterial duct, thereby improving pulmonary blood flow and enhancing gas exchange.

SEA is supported by a grant from the British Heart Foundation. We thank Helen Braid, Richard Bass, and Jason Mutch for their assistance with the experimental work.

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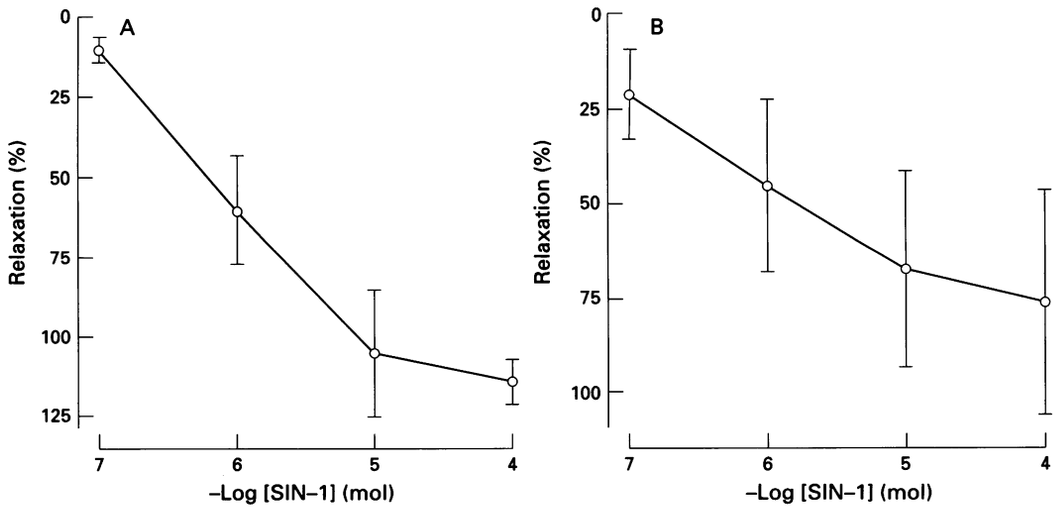

Figure 4 Force against time showing the percentage relaxation of the preconstricted state of (A) arterial ducts and (B) aortic rings to the nitric oxide donor SIN-1. All rings were preconstricted with 40 mmol/l of potassium.

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