The Response of the Lamb Ductus Venosus to Prostaglandins and Inhibitors of Prostaglandin and Thromboxane Synthesis

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SUMMARY. Rings of the umbilical end of the ductus venosus (the so-called sphincter) from near-term fetal and newborn (1-3 days of age) lambs were studied in vitro at low (17-31 mm Hg) and high (504-705 mm Hg) Po2. Tissues developed a modest contraction upon exposure to a high Po2; however, this contraction generally was not sustained and tended to be greater in the fetus than in the newborn. In contrast, excess potassium (55 mm) produced a sustained contraction whose magnitude was greater in the newborn. The cyclooxygenase inhibitor, indomethacin, contracted both the fetal and the neonatal ductus venosus; however, responses in the newborn were often not maintained. Furthermore, indomethacin enhanced the potassium response in the fetus, whereas it had no significant effect in the newborn. The thromboxane synthesis inhibitor, compound OKY-1581, was ineffective in either age group. Prostaglandin (PG) E2 and PG12 relaxed the ductus venosus, the former being slightly more potent. Conversely, stable PG endoperoxide analogs (9α,11α-epoxy-methano and 9α,11α-methano-epoxy compounds) and PGF2α were contractile agents. Endoperoxide analogs were more effective in the newborn than the fetus and their action consistently exceeded that of PGF2α. We conclude that the ductus venosus sphincter is endowed with functional muscle cells. These cells are under the influence of a relaxing product of the cyclooxygenase reaction which may be identified with either PGE2 or PG12. This prostaglandin-mediated relaxing mechanism may contribute to prenatal patency of the vessel. Postnatal closure of the ductus venosus is unlikely to be a direct effect of oxygen on the sphincter muscle. The prostaglandin endoperoxides, however, are well suited for playing a role in the latter process. (Circ Res 51: 580-586, 1982)
taglandins may also regulate the muscle tone of the ductus venosus. Our present findings support this possibility.

**Methods**

Experiments were performed on rings of the umbilical end of the ductus venosus (henceforth called the ductus venosus sphincter) from fetal or neonatal lambs (Suffolk or Suffolk-Dorset crossbreed). The gestational age of fetuses varied between 132 and 146 days (term, 147 days), whereas all but five newborns were used within 1 day (from 3 hours upward) of vaginal delivery. The remaining newborns were studied at 2 (four animals) or 3 (one animal) days of age.

**General Procedure**

**Fetus**

Pregnant ewes were anesthetized with intravenous pentobarbital (30 mg/kg), intubated, and thereafter were ventilated with a mixture of methoxyflurane, nitrous oxide, and air. Lambs were exteriorized and were placed, with the placental circulation intact and the head covered with a rubber glove, under an airtight hood. The hood was positioned close to the uterus and received a continuous inflow of nitrogen to keep the ambient Po2 at about 10 mm Hg. Animals were then killed by rapid exsanguination and the superior surface of the liver exposed. The capsule of the liver was incised and the hepatic veins were uncovered by gently scraping away the parenchyma with a moist sponge.

Following ligation and division of the veins, the ductus venosus was exposed and tied in proximity of its confluence with the inferior vena cava. The ductus, with a section of the liver attached, was then transferred into a dissection dish through which ice-cold Krebs solution preequilibrated with 5% CO2 in N2 was circulated. Flow rate was 60 ml/min to remove rapidly any liver constituent being released during dissection. The same solution was used to keep the tissues moist throughout the operative procedure in the animal. Before isolating the ductus sphincter, a glass rod (diameter, 1.3 mm) was inserted into the ductus at its ductus end and was advanced past the sinus end until the tip emerged from the liver. With this rod in place, it was possible to locate the portal sinus within the substance of the liver and hence trim down the block to a small size. For the final stage of the dissection, the glass rod was removed, the flow rate was reduced to 12 ml/min, and, with the aid of a dissection microscope, tissues bordering the sphincter were trimmed off, first from the sinus end, and then from the ductus end. Special care was used to completely remove liver tissue from the outer surface of the sphincter region; nonetheless, in some instances, the removal was incomplete. The resulting ring preparation containing the sphincter was suspended between platinum hooks in a 20-ml organ bath. The lower hook was part of a stationary glass rod, while the upper hook was connected to a force-displacement transducer (Grass FT-03C) by silk. The initial tension was adjusted to about 250 mg, and this value was extrapolated from the transmural pressure in vivo (Edelstone et al., 1978) by using the Laplace relationship for a thin-walled cylinder.

**Newborn**

Animals were anesthetized with intravenous pentobarbital (25 mg/kg) and were subsequently exsanguinated by cardiac puncture. The operative procedure was the same as in the fetus, except that surgery was performed in the open air and the glass rod could not be inserted in some preparations due to constriction of the sphincter (see Results). The initial load on the neonatal ductus was set at about 300 mg.

With both preparations, recording was isometric and was displayed on a Grass polygraph. The organ bath was supplied from several reservoirs, and a system of three-way valves allowed a rapid change from one perfusion fluid to another. The perfusion rate was approximately 2 ml/min and the fluid temperature was 37°C. Both the reservoir and organ bath were continuously bubbled with the required gas mixture. The N2-5% CO2 gas mixture was used when mounting the preparation inside the bath (Po2 5–10 mm Hg). Subsequent equilibration and drug tests were conducted with Krebs medium gassed with either 2.5% O2 and 5% CO2 in N2 (Po2 between 17 and 31 mm Hg; "low Po2") or 95% O2 and 5% CO2 (Po2 between 504 and 705 mm Hg; "high Po2"). The oxygen content of the medium was measured with an Instrumentation Laboratory gas analyzer.

**Solutions and Drugs**

The Krebs solution had the following composition (mm): NaCl, 118; KCl, 4.7; CaCl2, 2.5; KH2PO4, 1; MgSO4, 0.9; dextrose, 5.1; and NaHCO3, 25. Potassium-Krebs solution (20 or 55 mm) was prepared by substituting NaCl with an equimolar amount of KCl. The pH of the solution was 7.4 after equilibration with gas mixtures containing 5% CO2.

The following compounds were used: PGE2, PGF2a, sodium salt, PGF20, and the stable PG endoperoxide analogs, 9a,11a-methano-epoxy-15-hydroxy-prosta-5,13-dienoic acid and 9a,11a-epoxy-methano-15-hydroxy-prosta-5,13-dienoic acid (Upjohn); indomethacin (Sigma); sodium-(E)-3-[4-(3-pyridylmethyl)phenyl]-2-methacrylate (compound OKY-1581, Oha); dibenzylene hydrochloride (Smith, Kline and French); and procaine hydrochloride (Winthrop). PGE2, PGF2a, and PG endoperoxide analogs were dissolved in ethanol (1–5 mg/ml) and aliquots of the ethanol solution (stored at -20°C) were diluted with saline on the day of the experiment. PGI2 was dissolved directly in ice-cold Tris buffer (50 mm, pH 9) prior to use. Indomethacin was dissolved in ethanol (10 mg/ml) prior to preparation of the final solution in Krebs medium. With the exception of dibenzylene, all inhibitors were included in the Krebs solution bathing the ductus. Dibenzyline was added directly to the bath, allowed 10 minutes of contact with the tissue, and washed out. Prostaglandins were tested in sequential doses, using 3- to 10-fold increments in 50- to 200-μl volumes. Certain doses were often repeated twice or even three times to ascertain the consistency of responses. Doses of all compounds are given in molar concentrations and refer to their final concentration in the bath.

**Analysis of Responses**

Effects of contractile agents were measured by the increase in tension over the basal tension at the end of the equilibration period. To determine relaxant responses, tissues were contracted by exposure to high Po2 and indomethacin 2.8 × 10-4 M. In certain experiments, the contractile tension was increased further by raising the potassium content of the medium to 20 mEq/l. Data are expressed as the mean ± se. Statistical analysis of unpaired and paired data has been made using the Student's t-test or the Welch t-test if the variance ratio was significant. Multiple comparisons have been made with an analysis of variance and Duncan's multiple range test.
Results

Morphology of the Ductus Venosus Sphincter

When observed from the portal sinus, the fetal sphincter appeared as a circular or slightly oval opening limited by a ridge of tissue that was variably developed among animals. Occasionally, this ridge was incomplete or was indented by little perforations due to portal branches. The diameter of the lumen ranged between 1 and 4 mm (average about 2.5 mm) and showed no obvious correlation with the gestational age of the animal. The sphincter was narrower in the newborn (diameter <2.5 mm) and more frequently oval in shape, though findings were highly variable and seemingly unrelated to the age. In fact, in 1-day-old newborns, the size of the sphincter varied so widely to span over the full range between near closure and fetal-like patency. A 3-day-old newborn, on the other hand, still exhibited a well-dilated sphincter. In six newborns, the constricted ductus had an intramural hemorrhage which took the form of scattered patches or radial streaks extending from the base of the intraluminal ridge into the wall of the portal sinus. The ductus side of the sphincter, while resembling the sinus side in general shape, was never interrupted by branches to the substance of the liver and was also free from hemorrhagic lesions after birth.

Response to Changes in PO2 and Potassium

When first set up in the bath gassed with 5% CO2 in N2, the ductus venosus sphincter from fetal lambs either retained its basal tension or, more often, relaxed slowly. Exposure to a low PO2 resulted in a brisk contraction which started after brief delay (<2 minutes) and abated with variable speed (mean, 17 minutes; range, 4-44 minutes) despite the constant oxygen content of the medium (Fig. 1, top tracing). Once a stable baseline was attained, mean tension values were about 80% of those set initially. The ductus contracted again when the PO2 was raised to 504-705 mm Hg, though the contraction had a longer latent period (mean, 5 minutes; range, 1-11 minutes) (Fig. 1, middle tracing). However, this response did not differ in magnitude from that observed at low PO2 and, likewise, was not maintained in 33 of 41 preparations studied. Partial or complete reversal occurred over a 29- to 76-minute period. Furthermore, in certain experiments, the tension output of the ductus did not change upon transfer to either a low (seven experiments) or a high (four experiments) PO2. In contrast, excess potassium (55 mM) consistently produced a sustained contraction, regardless of the PO2 of the medium (Fig. 1, bottom tracing). Table 1 provides a summary of responses to oxygen and potassium in the fetus. The same table shows that the neonatal ductus usually failed to respond to a low PO2 and also tended to produce a smaller contraction to a high PO2 compared to the fetal ductus. Unlike the oxygen response, however, the potassium response was better developed in the newborn than in the fetus. In either age group, the contractile behavior of the preparation was not affected in any obvious manner by the presence of small residues of liver tissue on the vessel wall (see Methods).

Effects of Indomethacin and Compound OKY-1581

The fetal ductus developed a slow and progressive contraction when treated with the cyclooxygenase
inhibitor indomethacin at a concentration of 2.8 \times 10^{-6} \text{ M}. A maximum was attained in 12-80 minutes and the mean tension was about three times the control value (Table 1). This effect occurred at both low and high \text{PO}_2 and was sustained in all but three preparations. Likewise, the potassium response increased on the average 2-fold (Table 1). The neonatal ductus was also contracted by indomethacin at a high \text{PO}_2 (Table 1). However, this contraction abated partially or completely in 6 of the 14 preparations studied. Furthermore, the potassium response was not changed significantly by indomethacin in the newborn (Table 1). In an additional 1-day-old newborn, however, the potassium response was peculiarly small (96 mg) and increased 4-fold upon indomethacin treatment. This unique finding, possibly reflecting the persistence of fetal-type responsiveness, was not included in Table 1.

Although normally quiescent, the fetal ductus exhibited spontaneous activity after treatment with indomethacin at high \text{PO}_2. This effect occurred in 7 of the 34 preparations listed in Table 1. As shown in Figure 2, the spontaneous activity consisted of two basic patterns, i.e., slow contractions occurring at irregular intervals with or without fast activity superimposed, or regular phasic contractions at the rate of 1.5-4 per minute. Under the same conditions, the ductus from 1-day-old newborns was spontaneously active in only 1 of 14 experiments. Regardless of the pattern, spontaneous activity in the fetal ductus was not affected by treatment with either dibenzyline 5 \times 10^{-7} \text{ M} (two experiments) or procaine 10^{-5} \text{ M} (one experiment).

Unlike indomethacin, compound OKY-1581, a thromboxane synthesis inhibitor (Feuerstein and Ramwell, 1981), had no effect on the basal tone of the fetal (six experiments) and neonatal (two experiments) ductus at a concentration of 10^{-8} or 10^{-7} \text{ M}. Furthermore, the compound did not modify contractile responses of the fetal ductus to potassium (one experiment) and the 9α,11α-epoxy-methano endoperoxide analog (three experiments, see below).

**Effects of Prostaglandins and Prostaglandin Endoperoxides**

\text{PGE}_2 and \text{PGI}_2 relaxed the ductus sphincter in a dose-dependent manner. Their threshold concentrations varied among preparations; this notwithstanding, the fetal ductus was found to be more sensitive to \text{PGE}_2 than \text{PGI}_2 (Table 2). No such difference in sensitivity occurred in the newborn (Table 2), although, in both age groups, dose-response curves were steeper with \text{PGE}_2. Responses to either compound were immediate in onset, progressed rapidly to a maximum, and were fully reversible after washing the preparation. In most cases, application of a maximally effective concentration (\text{PGE}_2, about 10^{-9} \text{ M}; \text{PGI}_2, 10^{-8} to 10^{-7} \text{ M}) resulted in nearly complete or complete reversal of the active tension developed by the ductus. The pattern of relaxant responses was identical in the fetus and the newborn (1 and 2 days of age) and also showed no obvious change, depending on whether the intrinsic tone of the muscle was raised with indomethacin alone or with indomethacin plus excess potassium. Figure 3 provides a representative sequence of dose-dependent responses to \text{PGI}_2.

**Table 2**

Response of the Ductus Venosus Sphincter to Prostaglandins

<table>
<thead>
<tr>
<th>Compound</th>
<th>Sign of response</th>
<th>Fetus</th>
<th>Newborn (1-day-old)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Threshold</td>
<td>ED_{50}</td>
<td>Threshold</td>
</tr>
<tr>
<td>\text{PGE}_2</td>
<td>Relaxation*</td>
<td>0.1-1 \times 10^{-10}</td>
<td>2.3 \pm 0.9 \times 10^{-10}</td>
</tr>
<tr>
<td>\text{PGI}_2</td>
<td>Relaxation*</td>
<td>0.1-10 \times 10^{-9}</td>
<td>Not measured†</td>
</tr>
<tr>
<td>Endoperoxide analog</td>
<td>Contraction</td>
<td>3.3-1 \times 10^{-8}</td>
<td>Not measured†</td>
</tr>
<tr>
<td>\text{PGF}_{2α}</td>
<td>Contraction</td>
<td>1-3.5 \times 10^{-7}</td>
<td>Not measured†</td>
</tr>
</tbody>
</table>

Figures (range or mean ± SE) are molar concentrations in the organ bath. Number of experiments in brackets.

* To measure relaxant responses, the contractile tension of the ductus was raised with indomethacin either alone or in combination with excess potassium (see Methods).

† \text{ED}_{50} is not given due to either the lability of the compound which precluded a precise measurement (\text{PGI}_2) or the absence of a maximal response over the dose range tested (\text{PGF}_{2α}).

‡ 9α,11α-epoxy-methano analog.
The 9α,11α-epoxy-methano endoperoxide analog and PGF$_{2α}$ contracted the fetal ductus and their threshold concentration was the same at a low and a high P$_{O_2}$. As shown in Table 2, the endoperoxide analog was more potent than PGF$_{2α}$ and produced a maximal response at a concentration around $10^{-6}$ M. PGF$_{2α}$ action, on the other hand, was still submaximal at $10^{-5}$ M. Whether maximal or submaximal, contractile responses of the fetal ductus to the highest concentrations of the endoperoxide analog and PGF$_{2α}$ were greater than those to 55 mm potassium (Fig. 4). In fact, in four experiments, the endoperoxide analog ($10^{-6}$ M) was applied on tissues contracted by 55 mm potassium and raised the tension from a control value of 167 ± 45 mg to a value of 531 ± 58 mg ($P < 0.001$, paired $t$-test). A similar difference was observed in the 1-day-old newborn (Fig. 4) despite the increased effectiveness of potassium stimulation postnatally (Table 1). Accordingly, the maximal response to the endoperoxide analog was greater in the newborn than in the fetus, although threshold and ED$_{50}$ values were not significantly different (Table 2). No conclusion is possible on the efficacy of PGF$_{2α}$ in the fetal vs. the neonatal ductus; however, threshold concentrations were similar in the two age groups (Table 2). While contracting the ductus, the endoperoxide analog and PGF$_{2α}$ elicited phasic discharges of variable amplitude (Fig. 3). In certain cases, their contractile action progressed in two stages, i.e., an initial stage in which the tension output rapidly attained near maximal values and a secondary, slower stage with superimposed spontaneous activity. In two separate experiments, the 9α,11α-methano-epoxy analog was tested on the fetal ductus and proved to be as effective as the 9α,11α-epoxy-methano analog. Whether relaxant or contractile, ductal responses to the prostaglandins were not prone to tachyphylaxis.

**Discussion**

Although it has often been assumed that blood flow through the ductus venosus is conditioned by extrinsic factors (Dawes, 1968; Meyer and Lind, 1966; Edelstone et al., 1978), the present results suggest that this vessel is capable of autonomous regulation, at least in its sphincter region, and implicate prostaglandins in this process. Specifically, prostaglandins may play a role in the patency of the vessel prior to birth. The following findings are consistent with this possibility: (1) the ductus venosus sphincter is endowed with functional muscle cells; their collective tension output, though modest in absolute terms, is commensurate with normal values of transmural pressure at that site (Edelstone et al., 1978); (2) the fetal ductus venosus contracts upon treatment with indomethacin, which implies the intramural function of a cyclooxygenase product, or products, with relaxing action on muscle cells; in fact, this relaxing mechanism is strong enough to curtail the contractile response of the preparation to excess potassium; and (3) PGE$_2$ and PGF$_2α$ potently relax the ductus venosus; these compounds are generally assigned an important function in keeping vascular beds dilated, both prenatally and postnatally. Whereas there is evidence to implicate a prosta-
glandin in ductus venosus patency, the question of the mechanism of ductus closure remains unanswered. Nevertheless, several considerations are in order to analyze pertinent data and possibly pave the way for future investigations. We confirmed that the time of ductus venosus closure varies among animals (see Zink and Van Petten, 1980) and that the potential for significant shunting still occurs on the 3rd day after birth. However, in certain animals, the ductus venosus sphincter constricts early in the postnatal period, and this constriction is seemingly abrupt enough to produce hemorrhagic lesions. The occurrence of the latter is difficult to reconcile with the idea of closure resulting from a passive collapse of the vessel and suggests instead an active event. Among the possible contractile stimuli, the postnatal rise in oxygen tension is conceptually most appealing in view of its known importance to closure of the ductus arteriosus. The neonatal ductus venosus sphincter, though exposed to venous blood on the luminal side, could be subjected to oxygen stimulation through intramural vessels supplied by the hepatic artery. However, this possibility is not borne out by our findings. An alternative possibility is that closure results from the direct effect of a constrictor compound on the muscle of the sphincter. This hypothetical agent may be formed intramurally, may derive from the adjacent liver tissue, or may reach the ductus via the portal circulation. These multiple sources for the constrictor are not mutually exclusive and, in fact, any variation in the relative importance of local vs. distant release sites could explain individual differences in the time of ductus venosus closure. Against this background, our findings with the prostaglandin endoperoxide analogs, a class of compounds mimicking both the natural endoperoxides and thromboxane A₂, assume special importance. The endoperoxide action conforms in many ways to that expected for the postulated effector of ductus closure. Endoperoxide analogs are potent constrictors of the ductus venosus, exceeding in their action the potassium stimulation. Furthermore, the pattern of responses (i.e., the combination of a sustained contraction with phasic discharges) implies myogenic propagation of excitation and subsequent recruitment of muscle cells. The occurrence of synchronized activity should lead to a well-coordinated contraction and should also make the muscle more responsive to other stimuli (see Ljung and Stage, 1975), including stimuli from adrenergic nerves which are present in the tissue (Ehinger et al., 1968; F. Coceani, E. Cutz, and P. Olley, unpublished data). Last, endoperoxide action is more effective in the neonatal than the fetal ductus venosus. In this connection, it must be pointed out that, in the newborn, indomethacin often fails to produce a sustained contraction and is also without significant effect on the potassium response. The latter findings may suggest that relaxing product(s) of the cyclooxygenase reaction become less important postnatally. Our hypothesis leaves unsettled the question of whether the active product is a prostaglandin endoperoxide or thromboxane A₂. Findings with OKY-1581 argue against the occurrence of thromboxane A₂ synthesis in the vessel wall; however, this compound could be formed within the liver parenchyma (Pace-Asciak and Rangaraj, 1978).

An interesting issue emerging from this work concerns the differences between the ductus venosus and the ductus arteriosus with regard to the organization of the prostaglandin system and the response to oxygen (Coceani et al., 1978; Coceani and Olley, 1982). Unlike the ductus arteriosus, the ductus venosus contracts weakly to oxygen, and responses are normally not sustained. On the other hand, the ductus venosus is contracted by the endoperoxide analogs, which are virtually inactive on the ductus arteriosus. The two vessels share the sensitivity to indomethacin and, by inference, the dependence on a prostaglandin mechanism to remain patent in the fetus. However, PGE₂, but not PGF₂α, is thought to be important in the ductus arteriosus, whereas either, or both, compounds could play a role in the ductus venosus.

Our findings have important implications, both conceptually and practically. The demonstration of a functional sphincter in the ductus venosus, whose state may be controlled by products of arachidonate cyclooxygenase, confirms a general feature of vascular regulation in the fetus. Furthermore, the constrictor effect of indomethacin, if verified in vivo, represents an additional, potential complication of treatment with cyclooxygenase inhibitors during pregnancy. Conversely, the same inhibitors could be used in the
newborn to close a persistent ductus venosus causing hepatic insufficiency. PGE2 or PG12, on the other hand, may be useful to dilate the ductus venosus in patients requiring cardiac catheterization in the newborn period.

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