Hormonal Role of Adenosine in Maintaining Patency of the Ductus Arteriosus in Fetal Lambs

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The hypothesis that endogenously released adenosine plays an important role in maintaining patency of the fetal lamb ductus arteriosus was tested. The design of the study was (1) to determine the effect, if any, of exogenous adenosine on blood flow through the ductus arteriosus and (2) to evaluate the relationship among the partial pressure of oxygen in arterial blood, circulating endogenous plasma adenosine concentration, and the rate of blood flow through the ductus. When exogenous adenosine (5 μmoles) was administered during oxygen-induced ductal constriction, ductal blood flow increased from 101 ± 6 ml/min to 153 ± 4 ml/min ($p < 0.01$). When fetal blood adenosine concentrations were measured during nonventilation and ventilation with 100% oxygen, endogenous adenosine concentrations fell to less than one-half of the preventilation levels, *i.e.*, from 1.12 ± 0.17 to 0.49 ± 0.03 μM ($p < 0.01$). Finally, when fetal lambs were ventilated with increasing concentrations of oxygen (0%, 10%, 20%, 60%, and 100%) and measurements obtained simultaneously at each level, there was a significant monoexponential relationship among the rise in PO_2, the fall in plasma adenosine concentration, and the decrease in ductal blood flow. These data suggest that: (1) adenosine is a potent vasodilator of the lamb ductus arteriosus during oxygen-induced vasoconstriction; (2) fetal endogenous plasma adenosine levels fall significantly when PO_2 is increased; and (3) the fall in adenosine concentrations parallels a decrease in ductal blood flow. The findings suggest that the endogenous vasodilator adenosine plays an important role in maintaining ductal patency *in utero*.

INFANTS WITH CERTAIN FORMS of congenital heart disease are completely dependent on pulmonary blood flow through a patent ductus arteriosus. If the ductus arteriosus constricts despite ongoing hypoxemia, the result is rapid deterioration and death of an infant from congenital heart disease that would be otherwise amenable to surgical palliation or repair. The underlying mechanism involved in maintaining ductal patency *in utero* and closure at birth remains to be explained, despite the fact that prostaglandins are used clinically to dilate the ductus arteriosus in infants with pulmonary atresia or interrupted aortic arch. According to current views, the prostaglandins that relax the ductus arteriosus are formed intramurally and exert their action on the muscle cells.[1] This hypothesis is not supported by Clyman,[2] who reported that: (1) the isolated lamb ductus arteriosus needs an oxygen tension greater than that present *in utero* to produce prostaglandin E_2 (PGE_2); and (2) indomethacin has a negligible effect on the isolated ductus arteriosus incubated in a low oxygen environment, and the drug produces a significant contraction in rings incubated at a high PO_2 (680–720 mmHg). These observations would indicate that locally formed prostaglandins do not, in fact, contribute to the patency of the ductus *in utero*. Whether or not maternal circulating prostaglandins play an important role in the regulation of blood flow through the ductus arteriosus remains unknown. However, it is known that circulating prostaglandins in human infants remain markedly elevated during the first postnatal month,[3] long after the ductus has closed; this would suggest that circulating prostaglandins in the newborn do not contribute to ductal patency.

The concept that a low partial pressure of oxygen (PO_2) *in utero* results in the continuous release of vasodilators which relax the vascular smooth muscle is one in which the adenosine hypothesis might be easily implicated.[4] Adenosine is a potent endogenous vasodilator which is known to affect vascular smooth muscle contractility.[5-11] Since the wall of the ductus arteriosus is sensitive to changes in tissue partial pressure of oxygen, and adenosine has been shown to be an important factor in coupling tissue oxygen supply to oxygen

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demand by altering vascular smooth muscle tone in heart and brain, we proposed to test the hypothesis that endogenously released adenosine, in response to changes in whole-body oxygenation, mediates the changes in ductal blood flow seen following oxygenation. The corollary would be that an increase in oxygen with ventilation at birth results in a decrease in levels of circulating adenosine. Thus, a withdrawal of vasodilator would permit expression of vasoconstrictor tone, resulting in closure of the ductus arteriosus.

The design of the present study was: (1) to determine the effect, if any, of exogenous adenosine on blood flow through the ductus arteriosus in fetal lambs with an intact placenta under conditions of nonventilation, ventilation of the fetus with 100% nitrogen, and ventilation with 100% oxygen; and (2) to evaluate the relationship between the partial pressure of oxygen in arterial blood and circulating endogenous plasma adenosine concentrations and the rate of blood flow through the ductus arteriosus.

Methods

The experiments were carried out in 22 predated pregnant ewes (135 days). The ewes were anesthetized *via* induction with ketamine (22 mg/kg), maintained on 2% halothane, and placed in the supine position. All fetal surgical procedures were performed with 0.5% xylocaine for local anesthesia. After exposing the uterus through a lower midline incision, a fetal hind limb was located and exposed through a small (2–3 cm) uterine incision. Polyvinyl catheters were inserted into the artery and vein and advanced into the descending aorta and inferior vena cava, respectively. These catheters were flushed with saline containing heparin and exteriorized, and the uterine incision was closed. The catheters were used for sampling arterial and venous blood for PO_2 and oxygen saturation. The catheters were also used for administration of drugs as well as for collection of blood for measurement of plasma adenosine concentrations, as described by Manfredi and Sparks.[12]

A second 6-cm incision was made in the uterine wall over the left side of the fetal chest. The fetus was partially exposed and a rubber glove filled with saline was placed over the head to prevent spontaneous ventilation. After a left thoracotomy through the fourth intercostal space, the pericardium was incised over the pulmonary trunk from the main pulmonary artery to the ductus arteriosus. With minimal blunt dissection, polyvinyl catheters were inserted through purse string sutures into the aorta and pulmonary artery, just proximal and distal to the ductus arteriosus. These were connected to pressure transducers (Hewlett Packard Model 1280C®), which in turn were connected to an eight-channel Hewlett Packard recorder. An electromagnetic flow transducer (Biotronex 610®) which had been precalibrated *in vitro* was placed around the ductus arteriosus and connected to a Biotronex® flow meter to continuously monitor and record ductal blood flow. Flow transducers with internal diameters of 4.0 to 6.0 mm were required to provide good electrode contact with minimal vessel constriction. In rare instances, ductal constriction during oxygen ventilation was of sufficient magnitude as to prevent adequate electrode contact and, hence, preclude recording of ductal blood flow. No experiments of this nature were included in the data presented.

Zero flow determinations were made at regular intervals throughout the experiment by simultaneous occlusion of both the aortic and pulmonic ends of the ductus arteriosus. Aortic and pulmonary artery pressures and ductal flow were simultaneously monitored and recorded. Ductal resistance was calculated by dividing ductal perfusion pressure (PAP-AOP; or occasionally AOP-PAP, to yield positive resistance values) by flow (ml/min) and expressed as ductal resistance units (DRU). To evaluate the effect of exogenous adenosine on ductal resistance, control data were obtained, followed by intravenous bolus injections of adenosine (0.5 ml of 10 mM solution) *via* the hind limb venous catheter under the conditions of: (1) nonventilation; (2) fetus ventilated with 100% nitrogen; and (3) fetus ventilated with 100% oxygen. Arterial blood gases and changes in ductal resistance were recorded with each injection.

To evaluate the effect on endogenous plasma adenosine concentrations of nonventilation, ventilation with 100% nitrogen, and ventilation with 100% oxygen, a second series of experiments was conducted (N = 9). In the nonventilated fetus, 3-ml-blood aliquots were drawn from the hindlimb arterial and venous catheters. These blood samples were immediately placed in test tubes immersed in ice and containing 0.25 ml of 3 mM EHNA [erythro-9-2(2-hydroxy-3-nonyl)adenine] (Burroughs Wellcome) and 26 mM dipyridamole (Boehringer Ingelheim, Ltd.) to prevent deamination and cellular uptake of adenosine, respectively. An additional 1 ml of blood was drawn for blood gas determination, pH, and hematocrit. These 3-ml blood samples were spun immediately in a refrigerated centrifuge (5 C, 16,000 rpm, 10 min), after which 1.0 ml of plasma was withdrawn from each tube and added to 0.25 ml of 35% perchloric acid. These tubes were kept on ice until they were centrifuged (5 C, 16,000 rpm, 20 min). The acid extracts (supernatant fraction) were neutralized with KOH, filtered (millipore, 0.22 m), and stored at −20 C until the assays were performed.

After the blood samples had been collected from the nonventilated animal, the fetus was intubated and ventilated with 100% nitrogen. After an equilibration period

of 20 minutes, blood samples were again withdrawn from the artery and vein cannulas for adenosine levels and blood gas determinations. The plasma concentrations of adenosine under the two experimental conditions were then compared with plasma adenosine concentrations *in utero*.

A third series of experiments was conducted (N = 6) to evaluate the relationship among endogenous plasma adenosine concentrations, ductal blood flow, and arterial PO_2. After arterial and venous blood samples had been collected for measurement of adenosine levels, the fetus was intubated and ventilated with increasing concentrations of oxygen (0%, 10%, 20%, 60%, and 100%). After a 20-minute period of equilibration, blood samples were drawn at each level for measurement of hematocrit, PO_2, and plasma adenosine. Throughout the experiments, ductal blood flow was continuously monitored and recorded. Changes in plasma adenosine concentrations were correlated with changes in ductal blood flow and PO_2.

Blood Sample Analysis

Plasma adenosine was assayed by reverse-phase high performance liquid chromatography (Waters Associates, M-6,000-A pump, Model 440 Absorbance Detector®). One hundred μl of the sample were assayed using a 1-cm spherisorb-5-RP_{18} guard column and an ultrasphere-5-C_{18} analytical column with a single mobile phase (isocratic elution method) consisting of a 4 mM KH_2PO_4 buffer, pH 4.6 with 5% methanol, and a flow rate of 1.5 ml/min. The UV absorbence of the sample was monitored at 254 nm and recorded on a strip-chart recorder. The precision of the assay was determined by repetitive sampling of individual blood samples (variance = 10%). Exogenous adenosine was added to blood samples containing dipyridamole and EHNA, and mean recovery was determined to be 85 ± 2%. Reported values were not corrected for these losses. Adenosine peaks were identified by comparing the retention time of the sample to known standards, and the disappearance of the peak when treated with adenosine deaminase. The peaks were quantified using peak height and external standards. Final plasma concentration of adenosine was determined by accounting for hematocrit and dilutional factors.

Data Analysis

Each animal served as its own control. Statistical analyses were based on the t-distribution for paired data (experimental *versus* control), and by the analysis of variance (ANOVA). The relationships among ductal flow, adenosine concentration, and PO_2 were evaluated using curve-fitting analysis, using a least squares analysis. A p value of 0.05 was considered significant.

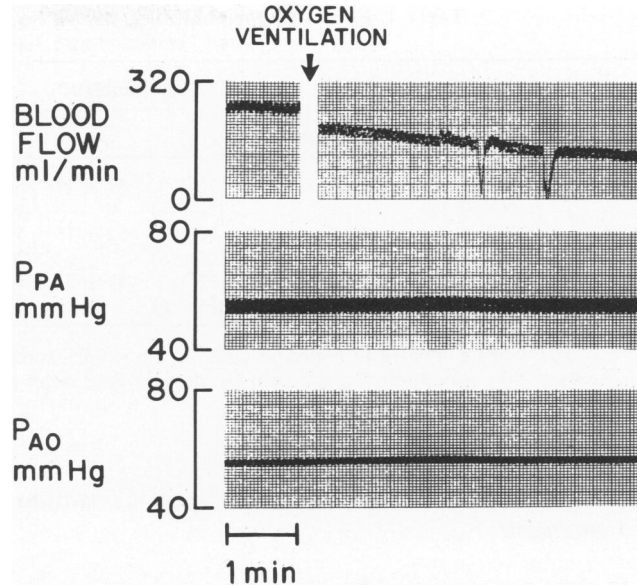

FIG. 1. The effect of 100% oxygen ventilation on the ductus arteriosus *in utero*. The decrease in blood flow is proportional to an increase in ductal resistance and reflects ductal vasoconstriction.

Results

Effects of Exogenous Adenosine

In seven fetal lambs with intact placentas, the mean PO_2 during nonventilation and ventilation with 100% nitrogen was 27.9 ± 1.3 mmHg and 23.9 ± 2.2 mmHg, respectively. During ventilation with 100% oxygen, the mean PO_2 was 129.9 ± 14.6 mmHg. This increase in PO_2 paralleled a marked decrease in ductal blood flow, from a mean of 213 ± 25 ml/min to 101 ± 6 ml/min ($p < 0.05$) (Fig. 1, Table 1). When adenosine (5 micromoles, IV bolus) was administered during nonventilation and ventilation with 100% N_2, ductal blood flow remained the same, *i.e.*, 213 ± 25 ml/min *versus* 207 ± 26 ml/min and 210 ± 20 ml/min *versus* 204 ± 19 ml/min, respectively (Fig. 2, Table 1). Although the adenosine injection was associated with a fall in the mean aortic and pulmonary artery pressure, the pressure gradient across the ductus remained the same. When the fetus was ventilated with 100% oxygen, arterial PO_2 increased from a mean of 27.9 ± 1.3 to 129.9 ± 14.6, and ductal blood flow fell from 213 ± 25 to 101 ± 6 ml/min (Table 1). The injection of adenosine resulted in an abrupt increase in flow from a mean of 101 ± 6 to 153 ± 4 ml/min ($p < 0.05$) (Fig. 3, Table 1). This occurred despite a fall in mean aortic and pulmonary artery pressures similar to that observed during adenosine infusion *in utero*. The adenosine produced a decrease in ductal resistance from a mean of 0.106 ± 0.034 to 0.059 ± 0.018 DRU (Table 1).

TABLE 1. *Effects of Exogenous Adenosine on Fetal Ductal Blood Flow During Nonventilation* (in Utero) *and Ventilation with 100% Nitrogen and 100% Oxygen*

	PO_2 (mmHg)		PA_p (mmHg)		Ao_p (mmHg)		DBF (ml/min)		DR	
	C	Ado	C	Ado	C	Ado	C	Ado	C	Ado
In Utero	27.9 ± 1.3 (N = 7)	27.9 ± 1.2	70 ± 1 (N = 6)	51 ± 1*	66 ± 1 (N = 6)	48 ± 1*	213 ± 25 (N = 6)	207 ± 26	0.022 ± 0.002 (N = 6)	0.020 ± 0.004
100% N_2	23.9 ± 2.2 (N = 10)	23.6 ± 2.1	70 ± 1 (N = 5)	52 ± 1*	59 ± 2 (N = 5)	44 ± 1*	210 ± 20 (N = 5)	204 ± 19	0.057 ± 0.007 (N = 5)	0.045 ± 0.005
100% O_2	129.9 ± 14.6 (N = 6)	129.0 ± 14.0	57 ± 3 (N = 6)	47 ± 3*	60 ± 6 (N = 6)	47 ± 5*	101 ± 6† (N = 6)	153 ± 4*	0.106 ± 0.034 (N = 6)	0.059 ± 0.018

* $p < 0.01$, when compared to respective controls; † $p < 0.05$, compared to *in utero* blood flow.
C, control; Ado, adenosine; N_2, nitrogen; O_2, oxygen; PA_p, mean pulmonary artery pressures; Ao_p, mean aortic pressure; DPF, mean ductal blood flow; DR, ductal resistance units.
Data are expressed as mean ± SEM.

Plasma Adenosine Levels During Different Conditions of Ventilation

In eight nonventilated fetal lambs, arterial and venous plasma adenosine concentrations were 1.12 ± 0.17 and 1.06 ± 0.07 μM, respectively. Similar arterial and venous concentrations were noted during ventilation with 100% nitrogen, (*i.e.*, 1.03 ± 0.08 and 0.98 ± 0.09 μM, respectively). When the fetal lambs were ventilated with 100% oxygen, the arterial and venous plasma adenosine concentrations fell to less than one-half the preventilation values, respectively (*i.e.*, 0.49 ± 0.03 and 0.52 ± 0.06 μM) (Fig. 4). This decrease in plasma adenosine concentration was associated with a marked increase in PO_2.

Effect of a Stepwise Increase in Arterial PO_2 on Ductal Flow and Plasma Adenosine Concentration

In the third series of experiments (N = 6), the fetal lambs were ventilated with increasing concentrations of oxygen (0%, 10%, 20%, 60%, and 100%). As oxygen was

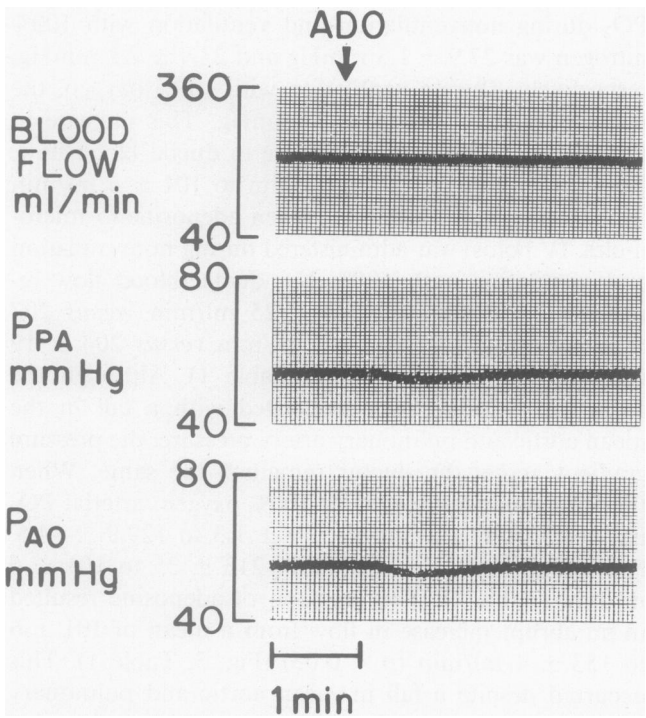

FIG. 2. The effect of adenosine on ductal blood flow in the nonventilated fetus. Adenosine (Ado) (5 μM, IV bolus) does not affect ductal resistance *in utero* (PO_2 23 mmHg).

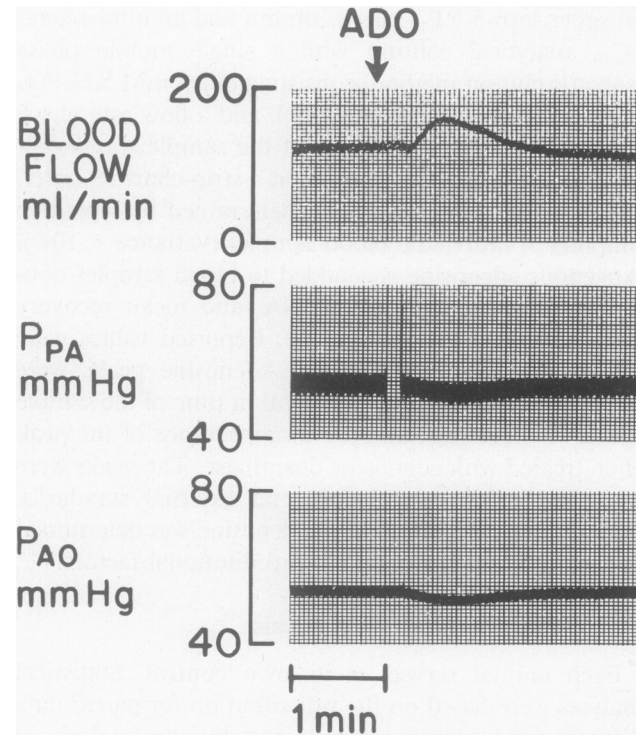

FIG. 3. The effect of adenosine on the ductus arteriosus during oxygen ventilation *in utero*. Adenosine (Ado) (5 μM, IV bolus) was administered during oxygen-induced vasoconstriction. The increase in mean ductal flow indicates active vasodilation.

increased from 0 to 100%, ductal blood flow decreased from a mean of 310.8 ± 32 ml/min to 84.16 ± 22.1 ml/min ($p < 0.05$, Table 2). Simultaneously measured, plasma adenosine concentrations decreased from a mean of 1.23 ± .13 μM to 0.51 ± .06 μM ($p < 0.05$) (Table 2). The increase in the fraction of inspired oxygen from 0 to 1.00 was associated with an increase in fetal arterial PO_2 from a mean of 19.4 ± 3.2 to 133.4 ± 34.8 mmHg, respectively (Table 2).

The relationship between plasma adenosine concentration and per cent of control ductal blood flow is shown in Figure 5. These data were analyzed using a curve-fitting program which provided a monoexponential fit of the data by a nonlinear least squares analysis. The line in Figure 5 is described by the equation:

$$y = \text{amplitude}\ (1 - e^{-kx}), \quad (r = 0.60,\ p < 0.01)$$

where x = concentration of plasma adenosine and y = % of control blood flow.

The relationship between PO_2 and plasma adenosine concentration is described in Figure 6. A monoexponential curve best described these data:

$$y = \text{amplitude}\ (e^{-kx}) + 0.3, \quad (r = 0.50,\ p < 0.01)$$

with x = PO_2 and y = concentration of plasma adenosine. These analyses suggest that a dose-response type of relationship exists between PO_2, adenosine concentration in plasma and ductal blood flow.

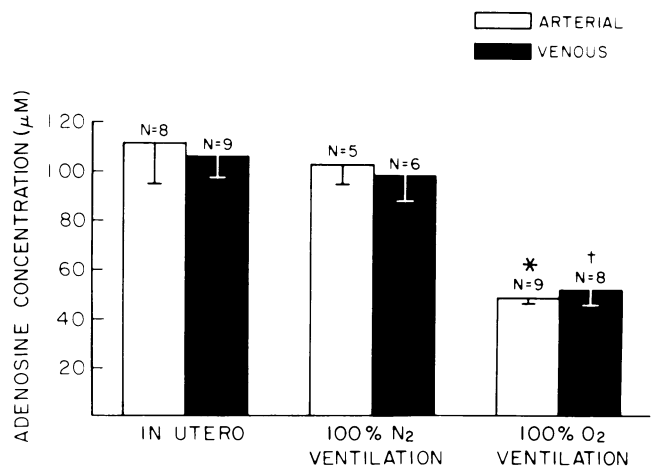

FIG. 4. Effect of ventilation on plasma adenosine concentrations in the fetal lamb. *$p < 0.01$ and †$p < 0.001$, compared to arterial and venous concentrations *in utero* and during 100% nitrogen ventilation.

Discussion

The adenosine hypothesis, as originally described in 1963 (Berne), demonstrated that tissue hypoxia was a potent stimulus for the formation and release of the vasodilator adenosine from intracellular adenine nucleotides. This concept provides the framework for the current study, in which we tested the hypothesis that a low PO_2 *in utero* is responsible for a constant release of adenosine which results in high circulating plasma adenosine concentrations, which in turn maintains the relaxation of ductal vascular smooth muscle. Conversely, at birth with ventilation, the improvement in tissue oxygenation would reduce adenosine release, lowering plasma concentrations, thus permitting the expression of vascular smooth muscle tone and closure of the ductus.

The major findings in these initial series of experiments demonstrate several points in support of this hypothesis. First, the ductus arteriosus responds to bolus injections of exogenous adenosine with a transient yet substantial

TABLE 2. *Effect of Increasing Concentrations of Inspired Oxygen on Plasma Adenosine Concentrations, Arterial PO_2, and Ductal Blood Flow in the Fetal Lamb*

		Ventilation (FiO_2)*				
	Nonventilation	II	III	IV	V	VI
	I (*In Utero*)	0	0.10	0.20	0.60	1.00
Plasma adenosine (μM)	1.42 ± 0.23	1.23 ± 0.13	0.86 ± 0.10	0.85 ± 0.12	0.59 ± 0.06	0.51† ± 0.06
Arterial PO_2 (mmHg)	32.70 ± 2.44	19.35 ± 3.28	25.20 ± 4.04	28.75 ± 2.60	55.10 ± 9.33	133.43† ± 34.84
Ductal blood flow (ml/min)	312.50 ± 29.0	310.83 ± 32.3	213.00 ± 29.0	217.50 ± 35.1	170.00 ± 33.6	84.16† ± 22.1

* FiO_2, fraction of inspired oxygen.

† Two-way analysis of variance and Duncan's test demonstrated significant differences at $p < 0.05$.

The results of ANOVA showed: (1) for plasma adenosine, the following columns are not different: I and II; III, IV, V, and VI; (2) for arterial PO_2, the following columns are not different: I, II, III, IV, and V; (3) for ductal blood flow, the following columns are not different: I and II; III and IV; III and V.

N = 6; data are expressed as mean ± SEM.

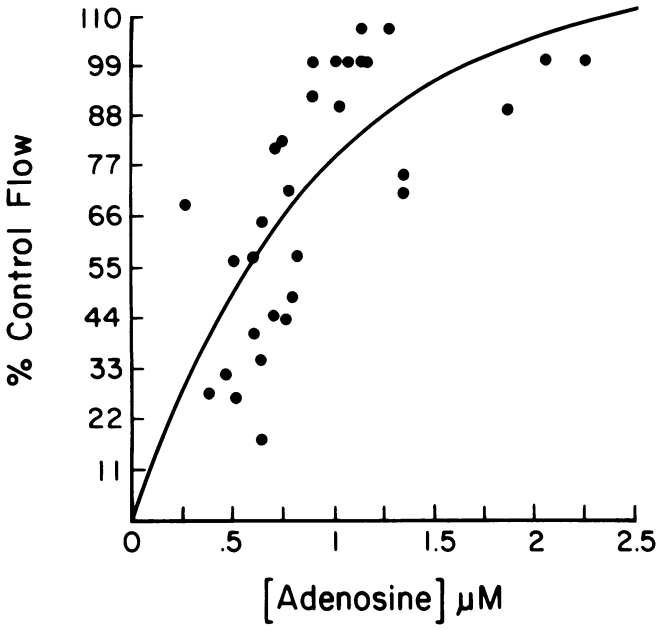

FIG. 5. The relationship between plasma venous adenosine concentration and the per cent of ductal blood flow is described by a monoexponential curve where $y = amplitude\ (1 - e^{-kx})$, $r = 0.60$, $p < 0.01$ (N = 6). The points represent individual values from all experiments.

dilation in the presence of ductal tone (with 100% O_2 ventilation). Second, circulating plasma adenosine concentration is elevated *in utero*, and is of the magnitude known to cause near maximal vasodilation in a variety of vascular beds ($1\text{–}2 \times 10^{-6}$ M). Third, endogenous

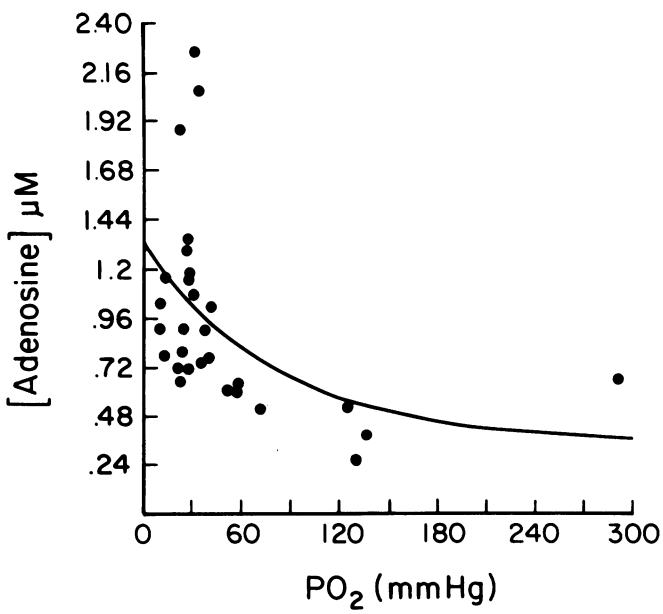

FIG. 6. The relationship between PO_2 (arterial) and plasma (venous) adenosine concentration is described by the monoexponential curve when $y = amplitude\ (e^{-kx}) + 0.3$, $r = 0.50$, $p < 0.01$ (N = 6). The points represent individual values for all the experiments.

plasma adenosine concentrations fall significantly in the fetus ventilated with 100% O_2. This fall parallels the increase in arterial PO_2 and the decrease in ductal blood flow, and the plasma adenosine concentrations reached are equivalent to those found in the newborn lamb with a closed ductus (unpublished observations) and in the adult dog.[11]

There is no information available on the role of adenosine in the regulation of ductal blood flow *in vivo*. However, in 1960 Kovalcik[13] reported that adenosine triphosphate (0.1 mM), adenosine monophosphate (1.0 mM), and adenosine (1.0 mM) caused a reversible inhibition of the contractile response to oxygen in isolated spiral strips of fetal lamb ductus arteriosus. The data of Kovalcik support the findings of the present study that adenosine can reverse the ductal constriction associated with elevated oxygen tensions.

As a previous report from our laboratory has shown,[11] measurements of plasma concentrations are difficult, in that the plasma pool is very labile due to the ability of the vascular endothelium and red blood cells to take up large amounts of adenosine and also due to the presence of adenosine deaminase which catalyzes the conversion of adenosine to the inactive metabolite inosine. Although we have taken precautions against these processes during the sample collection by the use of an uptake inhibitor (dipyridamole) and an inhibitor of adenosine deaminase, EHNA [erythro-9-2(2-hydroxy-3-nonyl)adenine], our studies (including the current study) have shown a relatively large interanimal variance. We interpret this as being caused by a true interanimal variance (as demonstrated by analysis of variance) and in part by the lability of the system. This makes it difficult to discriminate minor changes and necessitates major changes to establish statistical significance between conditions. However, in the present study the changes observed in plasma adenosine concentration in response to oxygenation (Table 2) were of sufficient magnitude not only to demonstrate significance, but also to represent a change in concentrations over the range known to be responsible for vasoactivity in whole organs as well as in vascular strips.[6–8]

The flow transduction system detects the directionality of flow and, in the fetus, flow is from right ventricle to pulmonary artery, across the ductus into the aorta (right to left shunt). A second important shunt occurs at the levels of the right atrium, across the foramen ovale to the left atrium, and then into the systemic circulation, although this shunt does not involve the ductus arteriosus. With O_2 ventilation, pulmonary resistance can decrease, thereby reversing the pressure gradient. Hence, flow across the ductus can go from aorta to pulmonary artery. This occasionally occurred in our preparation;

however, the data presented for ductal blood flow does not delinate the direction of flow as governed by changes in ductal resistance and plasma adenosine concentrations. The data presented in Table 2 and Figures 5 and 6 represent mainly venous plasma adenosine concentrations, in view of the fact that it is the venous blood that the ductus arteriosus is exposed to for the vast majority of measurements in our study. There were no significant differences between arterial and venous adenosine concentrations under the various conditions of this study (Fig. 4).

Table 2 represents the effect of a graded stepwise increase in the fraction of inspired oxygen on plasma adenosine concentrations, arterial PO_2, and ductal blood flow. It is noted that the means of the data do not change in a precise, graded, statistically significant manner with the fraction of inspired oxygen. This is not surprising, in that the means of the data are affected by interanimal and intraanimal variance and, more importantly, the degree of oxygenation at each level in each animal is influenced by a variety of physiological factors which govern oxygen exchange. However, the mean data in Table 2 do suggest that a relationship among plasma adenosine concentration, PO_2, and ductal blood flow exists. These relationships are best analyzed by relating each of the individual data points, and these data are shown in Figures 5 and 6. As both of these figures demonstrate, a significant correlation exists for PO_2 *versus* adenosine concentration and for adenosine concentration *versus* ductal blood flow (expressed as per cent of control). In both cases, a curve-fitting analysis provided a monoexponential fit of the data, suggesting that a significant relationship among these variables exists and that these relationships are similar to what one would predict for a dose-response interaction. These correlations do not prove cause and effect. However, the presence of adenosine in such high concentrations *in utero,* combined with the observation that the fall in plasma adenosine concentration with oxygenation is temporarily related to the closure of the ductus arteriosus, offers strong support for our hypothesis.

The concept that hypoxia *in utero* results in the continuous release of vasodilators which relax ductal vascular smooth muscle has developed as a result of the unsuccessful efforts to find a vasoconstrictor agent responsible for ductal closure after birth. Since the belief that the constriction of the ductus arteriosus is associated with an increase in blood PO_2 has been well-documented in several species *in vitro,* some investigators have directed their efforts to elucidating the direct effects of oxygen on ductal vascular smooth muscle. Fay[14] studied the effect of carbon monoxide on the response to oxygen in strips of ductus arteriosus obtained from neonatal guinea pigs. He reported that carbon monoxide inhibits oxygen-induced ductal contraction, and concluded that oxygen may have a direct effect by interacting with the cytochrome A_3 system, thereby increasing the rate of oxidative phosphorylation.

More recently, Coceani et al.[15] tested the effects of carbon monoxide and metyrapone on isolated ductus arteriosus preparations from mature fetal lambs equilibrated at low and high oxygen partial pressures. Their findings implicate a cytochrome P_{450}-catalyzed enzymic process in the contractile response of the vessel to oxygen. Whether oxygen has a direct effect on ductal vascular smooth muscle by producing an arachidonate monooxygenase metabolite and, thereby, producing ductal constriction remains to be determined.

With respect to specific circulating vasoconstrictive substances, Born et al.[16] reported that adrenalin and noradrenalin caused a reduction in ductal diameter in anesthetized lamb. Kovalcik[13] reported that noradrenalin, adrenalin, acetylcholine, histamine, and bradykinin caused the ductus arteriosus to contract in isolated spiral strips of sheep fetus ductus. Unfortunately, subsequent efforts to find a vasoactive substance which could be implicated in producing *in vivo* ductal constriction have been unsuccessful. The release of acetylcholine at birth causing ductal closure seems unlikely, since the ductal response to oxygen is not blocked by atropine. The effects of bradykinin seem to occur only in the presence of high concentrations of oxygen, and histamine has a minimal effect even in high oxygen tensions. Finally, blockage of alpha- and beta-adrenergic receptors does not abolish the response to oxygen. Hence, catecholamine release is an unlikely explanation for ductal closure at birth.

In summary, these findings suggest that an increase in oxygen with ventilation at birth results in a decrease in levels of circulating adenosine; thus, a withdrawal of the vasodilator may be, in part, responsible for the closure of the ductus arteriosus at birth. These data would suggest that the physiological role of adenosine may be expanded from the role of local regulator to that of circulating hormone in the immediate postpartum period. Further investigations are needed to develop a more detailed understanding of the actions of adenosine on the ductus arteriosus. With the limited usefulness of indomethacin for closing a patent ductus in premature infants, and the potential adverse effects of using prostaglandins to dilate the ductus arteriosus in infants with pulmonary atresia or interrupted aortic arch, it is important that the underlying mechanism involved in maintaining ductal patency *in utero* and closure at birth be elucidated if more definitive forms of therapy are to develop.

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