Effects of Acetylsalicylic Acid on the Ductus Arteriosus and Circulation in Fetal Lambs in Utero

MICHAEL A. HEYmann, M.D., AND ABRAHAM M. RUDOLPH, M.D.

SUMMARY  Intra-arterial and intravenous catheters were inserted in six fetal lambs at 125–130 days of gestation. On the following day, fetal arterial pressures and blood gases were monitored and fetal venous pressure-volume measurements in rats and the possibility that neural or humoral factors are immediately responsible for the venous changes. The possibility that the venous changes were the residual effect of neural or humoral vascular stimuli was not investigated.

THE GENERALLY has been thought that the ductus arteriosus is dilated passively during fetal life and actively constricted after birth. However, Coccoeini and Olley showed that in the presence of low P02, prostaglandins E1 and E2 produce active, dose-dependent relaxation of isolated strips of ductus arteriosus obtained from fetal lambs. They suggested that these prostaglandins may play a role in maintaining normal patency of the ductus arteriosus during fetal life.

To define the possible active physiological role of prosta-

glands in controlling the fetal circulation we studied the effects of inhibition of prostaglandin synthesis in fetal lambs in utero. This inhibition was produced by the administration of acetylsalicylic acid, which blocks prostaglandin synthetase, an enzyme system essential for production of prostaglandins.

Methods

We studied six pregnant ewes with time-dated gestational periods of 125–130 days. Under epidural anesthesia with tetracaine HCl, 20 mg, polyvinyl catheters were inserted into the maternal femoral artery and vein. The uterus was exposed through a midline incision and a small hysterotomy was performed. Catheters were inserted into a fetal hindlimb vein and artery and passed centrally into the inferior vena cava and abdominal aorta, respectively. An incision was made in the fetal neck through a second hysterotomy, and a
fetal carotid artery and jugular vein were cannulated. A catheter also was inserted through a purse-string suture in the esophagus and passed into the stomach. A left thoracotomy was performed and the main pulmonary trunk of the fetus was exposed. A catheter was inserted directly into the pulmonary trunk through a purse-string suture, and the chest was closed. A catheter was left in the amniotic cavity and the uterus and abdomen were closed. All catheters were filled with heparin and exteriorized to the flank of the ewe. Penicillin G (1 million U) and kanamycin (400 mg) were instilled into the amniotic cavity and the same doses were given intravenously to the ewe on the day of surgery and the following day.

Studies were performed on the day after surgery with the ewe standing quietly in her cage. Fetal carotid, femoral (central aortic), and pulmonary arterial blood pressures and intra-amniotic pressure were measured continuously with Statham P23Db pressure transducers and recorded on a Beckman type R direct-writing recorder. We corrected all fetal pressures, using the intra-amniotic pressure as a zero reference. Fetal heart rate was recorded from the arterial pressure tracing with a Beckman cardiomerometer.

When pressures and heart rate were stable, fetal arterial (central aortic) blood pH, Po2, and PCO2 were measured with Radiometer blood gas electrodes and a blood gas meter. We measured fetal cardiac output and its distribution by injecting radionuclide-labeled microspheres, 15 μm in diameter, into the jugular and femoral veins.6-9 Reference samples were withdrawn from the fetal carotid, femoral, and pulmonary arterial catheters and the volume of blood withdrawn was replaced with maternal blood.

Acetylsalicylic acid suspended in 5 ml of normal saline then was injected into the fetal stomach in doses ranging from 55 to 90 mg/kg of estimated fetal weight.10 Fetal pressures and heart rate were monitored continuously. We considered the development of an increased pressure difference between the pulmonary artery and central aorta to be indicative of constriction of the ductus arteriosus. When the maximal increase in pulmonary arterial pressure produced by acetylsalicylic acid had occurred, samples were obtained for determinations of arterial blood gas and pH, as well as of serum salicylate levels. We again measured fetal cardiac output and its distribution by injecting a second pair of microspheres with different radionuclide labels. Serum salicylate levels could not be determined in the fetal lambs because of the presence of an unidentified interfering substance.

In two fetuses prostaglandin E2 then was infused into the jugular vein at a rate of 0.1 μg/min per kg of estimated fetal weight for periods of 2 and 3 minutes to assess whether the drug was capable of affecting the circulation.

The uterus again was exposed following administration of spinal anesthesia (tetraclaine HCl, 20 mg) to the ewe. The fetal thoracotomy was reopened and the pulmonary artery, ductus arteriosus, and aorta were observed directly. The fetus was given a total of 15-20 mg of sodium pentobarbital into a hindlimb vein and 10% buffered formalin was infused into the pulmonary arterial catheter and simultaneously poured into the left chest to fix the ductus arteriosus in situ. In one ewe with twins one fetus did not receive acetylsalicylic acid and was used as a control. The ewe was killed with an overdose of sodium pentobarbital and the fetus and placenta were removed. Rings of tissue were cut from the main pulmonary trunk, the ductus arteriosus, and the descending aorta and their diameters were determined to assess the amount of constriction of the ductus arteriosus.

The fetal organs and placenta were dissected, weighed, and processed for isotope counting as previously described.7 We calculated cardiac output (combined ventricular output), organ blood flow, left and right ventricular outputs, and ductus arteriosus flow, using the concentrations of microspheres in each of the withdrawal samples as previously described.7-9 An estimate of the resistance to flow across the ductus arteriosus was made for the six animals by means of a paired t-test, with each fetus serving as its own control. When the level of significance by t-test was borderline and the data were asymmetric the Wilcoxon signed rank test was applied. All values given under Results are expressed as the mean ± SEM unless otherwise indicated.

Results

FETAL ARTERIAL PRESSURES AND HEART RATE

Pulmonary arterial blood pressure increased in each fetus (Fig. 1). The average time after administration of acetylsalicylic acid for pressure to reach the highest level was 58 minutes (range, 40–80 minutes) and the pressure elevation was maintained in all fetuses until the studies were terminated after about 2 hours or until prostaglandin E2 was administered. Central aortic blood pressure also increased, but to a lesser extent (Fig. 1). The increases in pulmonary arterial and central aortic systolic, mean, and diastolic blood pressures were significant statistically (Table 1). Pulmonary arterial pulse pressure increased from 23 ± 1.7 mm Hg to 37.5 ± 1.1 mm Hg (P < 0.005), but central aortic pulse pressure did not change. The difference in mean blood pressure between the pulmonary trunk and the aorta (PA–Ao) was 2 ± 0.3 mm Hg during the control period but rose to 11.2 ± 1.6 mm Hg after administration of acetylsalicylic acid (P < 0.005). This marked separation of pressures is shown in Figures 1 and 2.

Fetal heart rate was not significantly altered, averaging 200 ± 11.3 beats/min during the control period and 211 ± 10.0 beats/min after administration of acetylsalicylic acid.

BLOOD GASES

Control arterial blood pH (7.34 ± 0.02) and PCO2 (46 ± 1.4 torr) were normal in all fetuses.10 Arterial blood PO2 was normal in five fetuses but was reduced to 12 torr in one. The average for the six fetuses was 18 ± 1.7 torr. Since the responses of the fetus with the low PO2 were similar to those of the other five, the data from all six were pooled. After administration of acetylsalicylic acid there were no significant changes in arterial blood gases. At the time of maximal pressure effect, arterial blood pH averaged 7.33 ± 0.03; PO2, 17 ± 2.0 torr; and PCO2, 46 ± 0.9 torr.
CARDIAC OUTPUT (COMBINED VENTRICULAR OUTPUT) AND ITS DISTRIBUTION

A sufficient number of microspheres was present in the reference samples and all organs counted to permit accurate determination of blood flow. As shown in Figure 3, when expressed as a percentage change from control, blood flow to the placenta, adrenal glands, brain, heart, and lungs all increased significantly and hepatic arterial flow to the liver decreased significantly. Flow to the small and large intestines, kidneys, and upper and lower body decreased, although not significantly. The proportion of cardiac output (combined ventricular output) distributed to the placenta, adrenals, heart, and lungs increased but only that to the heart and lungs changed significantly. The proportion of cardiac output distributed to the brain decreased, although not significantly, whereas that to the liver, small and large intestines, kidneys, and upper and lower body decreased significantly.

Cardiac output (combined ventricular output) (Fig. 4) increased from 934 ± 55 ml/min during the control period to 1,112 ± 139 ml/min. When expressed in relation to fetal weight, cardiac output (combined ventricular output) was 456 ± 45 ml/min per kg during the control period and rose to 516 ± 18 ml/min per kg. Left ventricular output rose from 372 ± 25 to 499 ± 93 ml/min, and right ventricular output from 561 ± 55 to 613 ± 74 ml/min. Flow across the ductus arteriosus fell by 15.9 ± 3.4% (P < 0.01) from 495 ± 44 ml/min to 409 ± 20 ml/min (P < 0.025). The proportion of right ventricular output passing through the ductus arteriosus from 88.5 ± 1.3% to 69.7 ± 5.3% (P < 0.01).

RESISTANCE

The calculated resistance across the ductus arteriosus rose from 4.2 ± 0.5 units to 27.4 ± 4.01 units (P < 0.005).

INFUSION OF PROSTAGLANDIN E, DURING PULMONARY HYPERTENSION

Prostaglandin Ep produced a fall of 25 mm Hg in pulmonary arterial systolic and 12 mm Hg in mean pulmonary artery pressures within 1 minute after onset of infusion; within 2 minutes these had returned to normal levels. Central aortic pressure was unchanged. At 14 and 19 minutes, respectively, after the infusion was stopped the pulmonary arterial pressures again reached the high levels produced by the acetylsalicylic acid.

ANATOMY

When observed directly in the living fetus, the ductus arteriosus was markedly constricted and shortened in all six studies. However, in the control twin fetus that had not received acetylsalicylic acid only slight constriction was present; this probably was due to manipulation during dissection for exposure. During the past 4 years we have performed a thoracotomy for various experimental preparations on over 100 fetal lambs of different gestational ages and in all the ductus arteriosus has been widely dilated, with an external diameter similar to that of the main pulmonary trunk. The marked constriction of the ductus arteriosus

TABLE 1  Pulmonary Arterial (PA) and Central Aortic (Ao) Blood Pressures during the Control Period and at the Time of Acetylsalicylic Acid-Induced Maximal Pressure Increase in Six Fetal Lambs

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>After acetylsalicylic acid</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Systolic</td>
<td>Diastolic</td>
</tr>
<tr>
<td>PA</td>
<td>63.3 ± 1.1</td>
<td>40.3 ± 1.0</td>
</tr>
<tr>
<td>Ao</td>
<td>61.3 ± 1.2</td>
<td>38.8 ± 0.4</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM.
* P < 0.001.
† P < 0.005.
‡ P < 0.02.
ASPIRIN EFFECT ON DUCTUS ARTERIOSUS/Heymann and Rudolph

FIGURE 3 Percentage changes (±SEM) from control of organ blood flows after the administration of acetylsalicylic acid. P values refer to level of significance of change.

Discussion

It generally is believed that an increase in the concentration of oxygen to which the ductus arteriosus is exposed after birth is responsible for its closure. The exact mechanisms responsible for constriction of the smooth muscle in the wall of the ductus arteriosus are not clear nor is the role of various vasoactive substances which may be released into the bloodstream at the time of birth. Prostaglandins, a group of naturally occurring vasoactive lipids, require oxygen to produce smooth muscle constriction in rat stomach as well as in other organs. This fact led Coceani and Olley to consider the possible role of prostaglandins in the postnatal constriction of the ductus arteriosus. They studied isolated strips of ductus arteriosus obtained from near term fetal lambs and found that prostaglandins E₁ and E₂ had little or no effect on the ductus arteriosus after it was exposed to oxygen, but relaxed the anoxic ductus arteriosus. They suggested that prostaglandins E₁ and E₂ may play a role in maintaining the normal patency of the ductus arteriosus during fetal life. Elliott and Starling showed that prostaglandin F₁α produced constriction of strips of ductus arteriosus in near term fetal calves. Unlike prostaglandins E₁ and E₂, which exerted their effect in a low oxygen environment, the constrictor response to prostaglandin F₁α was accentuated in the presence of a high PO₂. The addition of a prostaglandin synthetase-blocking agent, indomethacin, to the tissue bath reduced the contractions produced by oxygen alone or by a combination of oxygen and prostaglandin F₁α. This suggested that certain of the endogenous biosynthesized prostaglandins might be involved in closure of the ductus arteriosus in response to an increase in oxygen environment. Since fetal arterial blood PO₂ is 18–25 torr, prostaglandins E₁ and E₂ probably would relax the ductus arteriosus in the normal fetus in utero. Blockade of their production might result in suppression of the dilator effect and constriction of the ductus arteriosus. Coceani et al. showed that inhibition of prostaglandin synthesis produced a gradual contraction of circular strips of fetal lamb ductus arteriosus at low PO₂ levels (<14 torr). Sharp et al. showed that indomethacin given to pregnant rats or rabbits near term produced constriction of the ductus arteriosus in the fetuses in utero. They used the whole body rapid freezing technique and obtained anatomical but no physiological data. We performed studies on intact fetal lambs in utero whose blood gases were normal and thus had the opportunity to study the...
effects of inhibition of prostaglandin synthesis on the vascular smooth muscle and circulation of the normal fetus.

After a latent period of about an hour, because of the intrauterine route of administration and the time taken for absorption of the acetylsalicylic acid, there was a marked constriction of the ductus arteriosus. This was shown by the increase in pulmonary arterial blood pressure, the significant fall in flow across the ductus arteriosus, and the marked increase in calculated resistance across the ductus arteriosus. Pulmonary arterial blood pressure fell immediately during the infusion of prostaglandin E₂, indicating that the effects noted were due to inhibition of prostaglandin synthesis rather than to a nonspecific effect of the acetylsalicylic acid.

It also is apparent from our studies that many fetal vascular beds are affected by prostaglandins and that circulation to the various organs can be altered by inhibition of prostaglandin synthesis. Previous studies have shown that prostaglandins E₁ and E₂ produce an increase in pulmonary blood flow,²⁰ and inhibition of their production would decrease this flow. Thus, it is likely that the increase in pulmonary blood flow we observed was due to constriction of the ductus arteriosus and to the significant increase in pulmonary arterial blood pressure.

In young calves prostaglandins E₁ and E₂ produce systemic hypotension, whereas prostaglandin F₂α causes hypertension.²¹ Inhibition of prostaglandin synthesis produced systemic hypertension and an increase in umbilical blood flow in each of the six fetuses in our studies. It is likely that this systemic hypertension resulted from the inhibition of the normal synthesis of prostaglandins E₁ and E₂ in the fetuses. The increase in umbilical blood flow can be explained by an increased perfusion pressure due to the systemic hypertension and also by a direct effect of acetylsalicylic acid on the umbilical circulation, since Novy et al.²² showed that administration of prostaglandin E₂ increased umbilical vascular resistance. Furthermore, prostaglandin E₁ has been shown to produce cerebral vasodilation²³ and in our studies synthetase inhibition produced an increase in cerebral blood flow.

It is of interest that, aside from the increase in pulmonary blood flow, all other changes in organ blood flows after acetylsalicylic acid administration were similar to those we have described during hypoxemia in fetal lambs.²⁴ Since the synthesis of prostaglandins requires oxygen it is possible that with significant deprivation of oxygen prostaglandin synthesis is inhibited and the circulatory effects of this could be quite similar to those produced by inhibition of prostaglandin synthesis by acetylsalicylic acid.

These studies have demonstrated that the ductus arteriosus, as well as peripheral vascular beds, of the 125- to 130-day fetal lamb are affected by inhibition of prostaglandin synthesis. It thus is probable that prostaglandins are involved in regulation of blood flow through the ductus arteriosus and the peripheral vascular beds in the normal resting fetus. Prostaglandin F₂α, which exerts greater effects at high levels of PO₂,²⁴ probably plays little active physiological role in the fetus and only assumes importance in constricting the ductus arteriosus after birth. The effects of prostaglandins E₁ and E₂ are more prominent at the low levels of PO₂ normally present in the fetus and thus production of these prostaglandins in the ductus arteriosus may be important in maintaining its patency during fetal life. It is possible that this dominance of prostaglandin E₁ and E₂ activity, with a lack of prostaglandin F₂α activity, is responsible for maintenance of persistent patency of the ductus arteriosus in prematurely born human infants. Prostaglandin synthetase inhibition therefore may play an important role in therapeutic attempts at closing the ductus arteriosus in these infants.

Acknowledgments

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