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Temporal Response of the Fetal Pulmonary Circulation to Pharmacologic Vasodilators (42642)

FRANK J. ACCURSO AND RANDALL B. WILKENING

Division of Perinatal Medicine, Department of Pediatrics, University of Colorado School of Medicine and the Webb–Waring Lung Institute, Denver, Colorado 80262

Abstract. To determine the temporal response of the fetal pulmonary circulation to pharmacologic vasodilators and to assess vasoreactivity following vasodilation, we infused either acetylcholine, histamine, or bradykinin directly into the left pulmonary artery of 21 chronically prepared fetal sheep. Blood flow (Q) to the left lung was measured by electromagnetic flow transducer. Left pulmonary artery infusion of acetylcholine at 1.5 $\mu g \cdot min^{-1}$ for 2 hr produced an increase in Q from 59 ± 8 $ml \cdot min^{-1}$ to a peak of 113 ± 10 $ml \cdot min^{-1}$ at 20 min into the infusion ($P < 0.001$). After the peak at 20 min, Q steadily declined toward baseline to 66 ± 7 $ml \cdot min^{-1}$ at the end of the 2-hr infusion period ($P < 0.01$). Q in the ½-hr period following infusion was significantly less than the baseline period (47 ± 6; $P < 0.04$) with no change in pulmonary artery pressure. Similar patterns were seen with 2-hr infusions of histamine (150 $ng \cdot min^{-1}$) and bradykinin (100 $ng \cdot min^{-1}$). After a 2-hr infusion of one of the agents, a repeat infusion with that agent or a different one resulted in a diminished response. We conclude that fetal pulmonary vasodilation in response to local infusion of acetylcholine, histamine, or bradykinin is not sustained over a 2-hr period, and that following 2-hr exposure to vasodilators, pulmonary vascular resistance is increased and pulmonary vasoreactivity to pharmacologic vasodilators is decreased.

The pulmonary circulation undergoes a transition from high to low vascular resistance at birth. The mechanisms of this transition are not completely understood but involve striking vasodilatory responses to ventilation, oxygenation, and prostanoids (1–3). Understanding the mechanisms that maintain high fetal pulmonary vascular resistance may be important to understanding both the normal transitional circulation and the abnormal transitional circulation seen in persistent pulmonary hypertension of the newborn (4).

We have previously observed that the vasodilatory response of the fetal pulmonary circulation to an increase in fetal PO_2 is not sustained over a 2-hr period, suggesting the existence of time-dependent mechanisms that maintain high pulmonary vascular resistance despite a continued vasodilatory stimulus (5). We wondered, therefore, whether the fetal pulmonary vascular response to pharmacologic vasodilators were also time dependent. In this report, we describe the temporal response of the fetal pulmonary circulation to three pharmacologic vasodilators (acetylcholine, histamine, and bradykinin). We also examine the effects of pretreatment with a given vasodilating agent on fetal pulmonary vasoreactivity, defined here as the response to the same or different vasodilating agents. We employed the chronically prepared fetal sheep model with a left pulmonary artery infusion catheter, thereby allowing observation of the response over several hours with minimal systemic effects.

Materials and Methods. *Animal preparation.* Twenty-one mixed-breed (Rambouillet–Columbia) ewes between 112 and 131 days gestation were fasted for 48 hr before surgery (term = 147 days). Under intravenous pentobarbital sedation and 1% tetracaine hydrochloride spinal anesthesia (10–12 mg), a hysterotomy was performed followed by a left thoracotomy in the fetus to expose the heart and great vessels. Polyvinyl catheters were then inserted into the left pulmonary artery (0.043 in. o.d.), main (preductal) pulmonary artery (0.054 in. o.d.), and left atrium (0.054 in. o.d.) by direct puncture following the placement of purse string sutures as previously described (5–9). In two fetuses a polyvinyl catheter (0.043 in. o.d.) was placed in the right pulmonary artery. A cuff-type electromagnetic flow transducer (Micron Instruments) was placed around the left

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pulmonary artery to measure blood flow. Flow transducers were precalibrated using saline infusion through the carotid artery of the adult sheep *in situ*.

A separate hysterotomy was used to place catheters (0.054 in. o.d.) in the fetal abdominal aorta and the inferior vena cava via a fetal pedal artery and vein. A catheter (0.054 in. o.d.) was placed in the amniotic cavity to measure pressure.

The catheters and flow transducer cables were led subcutaneously to an external flank pouch. The ewes recovered promptly from surgery and were standing in their individual pens within 6 hr. The ewes were allowed water and food *ad libitum*. Catheters were maintained by flushing 1–3 ml heparinized saline (35 μ/ml) daily.

Physiologic measurements. Ewes were allowed at least 4 days recovery from operative stress prior to study. Pulmonary artery, aortic, left atrial, and amniotic catheters were connected to Gould Statham P23D pressure transducers. A cardiotachometer triggered by the pulmonary arterial signal-measured heart rate. Blood flow to the left lung (Q) was measured with an electromagnetic flow meter (Micron Instruments). Pressure, flow, and heart rate measurements were recorded on a Gould chart recorder. We considered the blood flow signal at end diastole as zero flow (6–10). This did not always coincide with the electronic zero of the flow meter. To confirm the reliability of estimating zero flow as the end diastolic flow signal, four fetuses were sacrificed while the blood flow was monitored as in previous studies (5, 9). The signal measured after fetal death was the same as the end diastolic blood flow tracing before death to within 20 ml/min, suggesting reliability of the method. Although absolute values for baseline measurements are uncertain, measurements of changes in flow are accurate.

Samples of blood (0.5 ml into heparin-coated capillaries) for pH, PCO_2, PO_2, and oxygen saturation measurements were drawn through the main pulmonary artery catheter and analyzed by means of a blood gas analyzer and hemoximeter (Radiometer, Copenhagen). The total amount of blood drawn for each study was no more than 6 ml over 3 hr.

Each fetus was studied over a 1-week period. On the final day of study each fetus and ewe was killed by separate injections of euthanasia solution (T61, Hoechst) at which time the condition and placement of the catheters and flow transducer were verified.

Drug preparation and delivery. Acetylcholine chloride (Sigma), histamine phosphate (Lilly), bradykinin (Sigma), dimeprit and 2-(2-pyridyl)-ethylamine (2-PEA, Smith Kline and French, Ltd., U.K.) were mixed fresh and diluted in saline on the day of each study. Precalibrated infusion pumps delivered the solutions at 0.1 ml $\cdot$ min^{-1}. The total fluid volume infused was less than 15 ml over 2 to 3 hr in each study.

Study design. Two sets of studies were done. In the first set (n = 18), we infused acetylcholine, histamine, or bradykinin to determine the temporal response to a given agent and to assess vasoreactivity upon repeat infusion of that agent. In the second set (n = 8), crossover studies were performed to determine the effect of a 2-hr infusion of one of the agents (acetylcholine, histamine, or bradykinin) on vasoreactivity defined as the response to 15-min infusion of another one of the agents. A total of 21 fetuses were studied with 5 fetuses studied as part of both sets of experiments. Infusion protocols were randomized for fetuses studied on more than one occasion. All studies were done on different days.

I. Infusion of acetylcholine, histamine, or bradykinin: A. Acetylcholine. After a control period of $\frac{1}{2}$ hr, acetylcholine at 1.5 μg $\cdot$ min^{-1} was infused through the left pulmonary artery catheter for 2 hr in nine fetuses. After 2 hr, the infusion was stopped and measurements were taken for an additional $\frac{1}{2}$ hr. This dose, which corresponds to 0.5 μg $\cdot$ kg $\cdot$ min^{-1} assuming an estimated fetal weight of 3 kg, was chosen because preliminary studies indicated that this dose produced a doubling of pulmonary blood flow without changing pulmonary artery pressure. To determine the effect of a 2-hr infusion of acetylcholine on a subsequent infusion of acetylcholine, in six of these fetuses a repeat infusion of acetylcholine for $\frac{1}{2}$ hr at the same rate with a fresh solution was begun 1 hr following the initial infusion.

In three fetuses acetylcholine infusion at

3.0 $\mu g \cdot min-1$ was done to examine the effect of a different dose on the temporal response to acetylcholine. To assess reproducibility of results, two fetuses received acetylcholine infusions on each of 3 consecutive days. Saline infusions at the same rate of infusion as the acetylcholine studies were performed in five fetuses to control for the effect of infusing 12 ml of saline over 2 hr. To confirm the local nature of the infusion, two additional fetuses received right pulmonary artery and main pulmonary artery infusions of acetylcholine at 1.5 $\mu g \cdot min^{-1}$.

B. Histamine. A similar experimental protocol was followed with histamine infused through the left pulmonary artery catheter at 150 $ng \cdot min^{-1}$ for 2 hr in nine fetuses with repeat infusion 1 hr following the initial infusion in six of these fetuses. This dose corresponds to 50 $ng \cdot kg \cdot min^{-1}$, assuming a fetal weight of 3 kg. Control infusions in the right pulmonary artery and the main pulmonary artery were done in two fetuses. Histamine infusion at 75 $ng \cdot min-1$ was performed in two fetuses to assess the temporal response to a different rate of histamine infusion.

As both H1 and H2 stimulation resulted in fetal pulmonary vasodilation separate infusions with dimeprit (1.0 $mg \cdot min^{-1}$), a selective H2 agonist, and 2-PEA (40 $\mu g \cdot min^{-1}$), a selective H1 agonist, were performed in one fetus (11).

C. Bradykinin. A similar experimental protocol was followed with bradykinin infused locally at 100 $ng \cdot min^{-1}$ in eight fetuses for 2 hr. Three fetuses had repeat infusions of bradykinin 1 hr following the initial infusion.

II. Effect of 2-hr infusion of histamine, acetylcholine, or bradykinin on the response to a different agent (crossover studies): A. Crossover studies with 2-hr infusion of histamine. After a $\frac{1}{2}$-hr baseline period acetylcholine at 1.5 $\mu g \cdot min^{-1}$ ($n = 5$) or bradykinin at 100 $ng \cdot min^{-1}$ ($n = 5$) was infused locally through the left pulmonary artery catheter for 15 min. After a recovery period of at least 15 min, histamine was infused at 150 $ng \cdot min^{-1}$ for 2 hr. Within an hour following discontinuation of the histamine infusion we performed a 15-min repeat infusion of acetylcholine or bradykinin at the same rate as the initial infusion. A 15-min infusion period was chosen because preliminary studies showed that Q achieved by 15 min into an infusion approximates the peak Q seen with continuous infusion, while following this abbreviated infusion period there is a rapid return to control values for Q and pulmonary vascular resistance. Four fetuses received 15-min infusions of histamine at 150 $ng \cdot min^{-1}$, acetylcholine at 1.5 $\mu g \cdot min^{-1}$, or bradykinin at 100 $ng \cdot min^{-1}$ 2 hr apart to test for the effect of a 15-min infusion of a given agent on a subsequent infusion of the same agent 2 hr later.

B. Crossover studies with 2-hr infusion of bradykinin. A similar experimental protocol was followed with 2-hr infusion of bradykinin at 100 $ng \cdot min^{-1}$. Histamine (75 $ng \cdot min^{-1}$) ($n = 3$) or acetylcholine (1.5 $\mu g \cdot min^{-1}$) ($n = 1$) was infused for 15 min before and after the bradykinin infusion. We choose a smaller dose of histamine than in the previous studies because preliminary studies had shown that larger doses of histamine resulted in greater peak blood flow that was not affected by the 2-hr infusion of bradykinin.

C. Crossover studies with 2-hr infusion of acetylcholine. A similar experimental design was followed for 2-hr infusion of acetylcholine at 3.0 $\mu g \cdot min^{-1}$. Histamine (75 $ng \cdot min^{-1}$) ($n = 3$) or bradykinin (100 $ng \cdot min^{-1}$) ($n = 1$) was infused for 15 min before and after the acetylcholine infusion. Doses were chosen through preliminary studies that indicated that higher doses of histamine led to much greater changes in pulmonary blood flow than produced by infusion of acetylcholine at 1.5 $\mu g \cdot min^{-1}$ and 2-hr infusion of acetylcholine then had no effect on repeat infusions of histamine.

Statistical analysis. Values for Q, mean pulmonary artery pressure (PAP), mean systemic arterial pressure (SAP), mean left atrial pressure (LAP), and the gradient between PAP and SAP (PAP-SAP), heart rate, and calculated PVR were taken every 10 min for the experiments with infusion of acetylcholine, histamine, or bradykinin alone and every 15 min for the crossover studies. Arterial blood gases were sampled every half-hour. Data were analyzed using repeated-measures analyses of variance with a commercially available statistics software

package on a VAX 1150 minicomputer (12). The method allows multiple comparisons through partition of the between-times sum of squares and the use of appropriate contrasts (13). Individual time points or periods in the study may be compared with other time points or periods. In those fetuses without left atrial catheters, left atrial pressure was assumed to be 3 mm Hg for the purpose of calculating pulmonary vascular resistance.

Results. *I. Responses to infusion of acetylcholine, histamine, or bradykinin: A. Acetylcholine.* Figure 1 illustrates the temporal response of PAP, Q, and PVR to infusion of acetylcholine in the left pulmonary artery. After an initial increase in Q, peaking at 20 min, Q declines steadily toward baseline and is not significantly different at 120 min from baseline. In the $\frac{1}{2}$-hr following acetylcholine infusion Q is significantly decreased ($P < 0.04$) and PVR is significantly elevated from the baseline period ($P < 0.01$). PAP did not significantly change. SAP was 41.4 ± 1.5 mm Hg ($n = 6$), PAP-SAP was 2.0 ± 0.6 mm Hg ($n = 6$), and LAP was 3.2 ± 0.5 ($n = 4$) during the baseline period and did not significantly change during the infusion or recovery period. Heart rate increased gradually during the infusion period to a peak of 185 ± 7 bpm at 90 min into the infusion from a baseline of 169 ± 6 ($P < 0.03$). Heart rate during the recovery period (173 ± 7 bmp) was not significantly different from baseline. PO_2 (n = 4) was 20.7 ± 0.6 mm Hg, PCO_2 was 44.4 ± 2.0 and pH was 7.38 ± 0.03 during the baseline period and did not significantly change throughout the study. The same pattern of an increase in Q peaking within the first 40 min of infusion and then a steady decline toward baseline was seen in all fetuses studied (gestational ages, 123–138 days). Baseline Q was correlated with gestational age ($r = 0.74$, $P < 0.02$) but peak Q achieved with infusion was not. There was no significant correlation between gestational age and the time to peak Q.

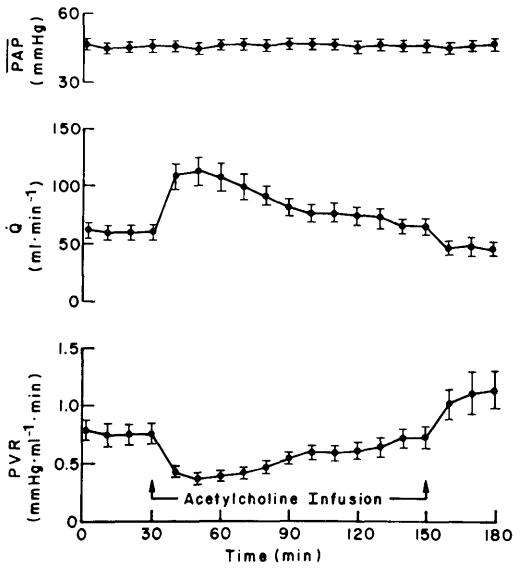

FIG. 1. PAP, Q, PVR are plotted for 2-hr local infusion of acetylcholine at 1.5 $\mu g \cdot min^{-1}$ through the left pulmonary artery of nine fetal lambs.

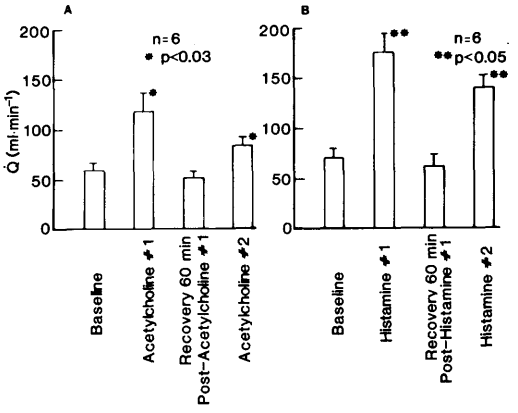

FIG. 2. (A) Q is shown for the baseline period, 20 min into a 2-hr left pulmonary artery infusion of acetylcholine at 1.5 $\mu g \cdot min^{-1}$ (ACH No. 1) and 20 min into a second infusion (ACH No. 2) begun 1 hr after the first. (B) Q is shown for the baseline period, 20 min into a 2-hr infusion of histamine at 150 $ng \cdot min^{-1}$ and 20 min into a second infusion begun 1 hr after the first.

Figure 2A illustrates the effect of 2-hr infusion of acetylcholine on a subsequent infusion of acetylcholine. Q increased significantly with the initial infusion of acetylcholine. A 1-hr recovery period followed. Q at the end of the recovery period was less than but not significantly less than Q in the baseline period. Repeat infusion of acetylcholine resulted in a significantly diminished response when compared to the first acetylcholine infusion. The increase in Q in response to the second infusion was signifi-

cantly less than the increase in Q in response to the first infusion ($P < 0.04$) in addition to the significant difference in the absolute levels of flow. PVR reflected the changes in Q.

Infusion at a 3.0 $\mu g \cdot min^{-1}$ in three animals increased Q from 48 ± 10 $ml \cdot min^{-1}$ to a peak of 129 ± 10 $ml \cdot min^{-1}$ occurring between 10 and 20 min. The same pattern of a decrease in Q toward baseline over the remainder of the infusion period was seen in each of these infusions. Repeat infusions of acetylcholine on consecutive days were highly reproducible. Saline infusions for 2 hr had no effect on pulmonary blood flow. Right pulmonary artery infusion of acetylcholine at 1.5 $\mu g \cdot min^{-1}$ had no effect on left pulmonary blood flow and main pulmonary artery infusion produced less than a 20% increase in left pulmonary blood flow.

B. Histamine. Figure 3 illustrates the temporal response of PAP, Q, and PVR for infusion of histamine in the left pulmonary artery. Q increases from a baseline of 58 ± 5 to a peak of 145 ± 17 $ml \cdot min^{-1}$ at 20 min and then declines steadily to 95 ± 9 $ml \cdot min^{-1}$ ($P < 0.01$), though still significantly greater than baseline ($P < 0.01$). PVR reflected changes in Q. PAP did not change. SAP was 43.3 ± 1.5 mm Hg ($n = 6$), PAP-SAP was 2.5 ± 0.5 mm Hg ($n = 6$), LAP was 2.6 ± 0.4 mm Hg ($n = 4$), and heart rate was 180 ± 10 bpm during the baseline period and did not change significantly during the infusion or recovery periods. PO_2 was 19.8 ± 1.5 mm Hg ($n = 4$), PCO_2 was 46.2 ± 0.6 mm Hg, and pH was 7.39 ± 0.01 during the baseline period and did not significantly change during the infusion or recovery periods. The same pattern of an increase in Q peaking within the first 40 min of infusion was seen in all animals studied (gestational ages, 118–140 days). Peak Q response to histamine was not significantly correlated with gestational age ($r = 0.62$, $P < 0.10$).

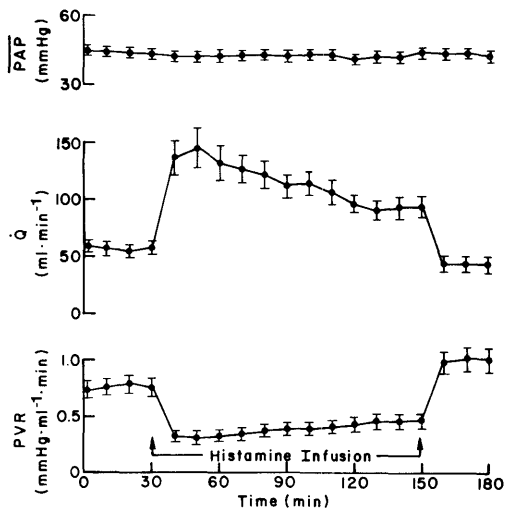

FIG. 3. PAP, Q, PVR are plotted for 2-hr local infusion of histamine at 150 $ng \cdot min^{-1}$ through the left pulmonary artery of nine fetuses.

Figure 2B illustrates the effect of a 2-hr infusion of histamine on a subsequent infusion of histamine. Q increased significantly with the initial infusion of histamine. A 1-hr recovery period followed. Q at the end of the recovery period was less than but not significantly less than Q in the baseline period. Repeat infusion of histamine resulted in a significantly diminished response when compared to the first histamine infusion. The increase in Q in response to the second infusion was significantly less than the increase in Q in response to the first infusion ($P < 0.05$) in addition to the significant difference in the absolute levels of Q.

Main pulmonary artery and right pulmonary artery infusion of histamine resulted in markedly diminished responses compared to left pulmonary artery infusion. Histamine at 75 $ng \cdot min^{-1}$ resulted in an increase of Q from 55 ± 10 to 113 ± 12 $ml \cdot min^{-1}$, peaking at 10–20 min into the infusion followed by a steady decline toward baseline.

Infusion of the H1 agonist 2-PEA in one fetus increased Q from 60 $ml \cdot min^{-1}$ to a peak of 130 $ml \cdot min^{-1}$ in the first 10 min, followed by a steady decline to 40 $ml \cdot min^{-1}$ at the end of the 2-hr infusion period. Local pulmonary infusion of the H2 agonist dimeprit in one animal increased pulmonary blood flow from 70 to 150 $ml \cdot min^{-1}$ at 30 min into the infusion. Q then steadily declined until the infusion was stopped at 60 min because the fetus became acutely hypotensive.

C. Bradykinin. Figure 4 illustrates the temporal response to bradykinin infusion into the left pulmonary artery. The response

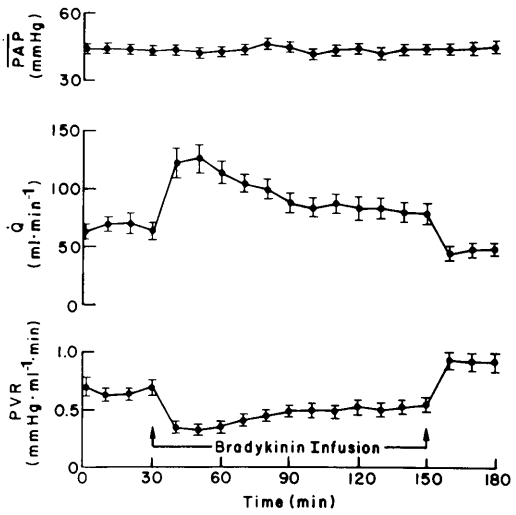

FIG. 4. PAP, Q, PVR are plotted for 2-hr infusion of bradykinin at 100 ng · min^{-1} through the left pulmonary artery of eight fetuses.

is qualitatively similar to that described for histamine and acetylcholine. SAP was 44.3 ± 1.8 (n = 8) mm Hg, PAP-SAP was 1.6 ± 0.5 mm Hg, heart rate was 173 ± 10 mmHg, and LAP was 2.8 ± 0.4 mm Hg during the baseline period and did not significantly change through the infusion or recovery periods. PO_2 was 18.3 ± 0.9 mm Hg in the baseline period (n = 4), PCO_2 was 44.5 ± 0.7 mm Hg, and pH was 7.40 ± 0.02 mm Hg during the baseline period and did not significantly change during the infusion period. Peak blood flow response to bradykinin was significantly correlated with gestational age ($P < 0.05$).

In three fetuses with repeat infusions of bradykinin 1-hr after an initial 2-hr infusion, peak Q for the first infusion was 104 ± 0.9 ml · min^{-1} followed by peak Q of 68 ± 7 ml · min^{-1} for the repeat infusion.

II. Crossover studies: A. Crossover studies with 2-hr infusion of histamine. The effect of a 2-hr infusion of histamine on the response to acetylcholine is shown in Fig. 5. Fifteen-minute infusion of acetylcholine at 1.5 μg · min^{-1} in five fetuses increased Q 128 ± 16 ml · min^{-1} from a baseline of 69 ± 5 ml · min^{-1}. Q following this infusion was 66 ± 7 ml · min^{-1} and not significantly different from baseline. Local infusion of histamine at 150 ng · min^{-1} for 2 hr resulted in a peak at 20 min (159 ± 14 ml · min^{-1}) and a steady decline toward baseline. Following the 2-hr infusion of histamine, Q was decreased at 54 ± 9 ml · min^{-1}. Repeat local infusion of acetylcholine at 1.5 μg · min^{-1} resulted in Q of 88 ± 12, significantly less than that of the first infusion ($P < 0.03$). The increase in Q in response to the second infusion of acetylcholine was significantly less than the increase in Q in response to the first infusion of acetylcholine ($P < 0.05$) in addition to the significant difference in the absolute level of Q. PAP did not change in these studies and PVR reflected the changes in Q.

Figure 6 illustrates the effect of 2-hr infusion of histamine on the response to infusion of bradykinin. Fifteen-minute infusion of bradykinin at 100 ng · min^{-1} in five fetuses increased Q to 122 ± 13 ml · min^{-1} from a baseline of 70 ± 10 ml · min^{-1}. Q following this infusion was 66 ± 6 ml · min^{-1} and not significantly different from baseline. Infusion of histamine at 150 ng · min^{-1} for 2 hr resulted in a peak Q at 20 min (150 ± 26 ml · min^{-1}) and a steady decline toward baseline. Following the 2-hr infusion of histamine, Q was decreased at 52 ± 9 ml · min^{-1}. Repeat local infusion of bradykinin at 100 ng · min^{-1} resulted in Q of 82 ± 14 ml · min^{-1}, significantly lower than the first infusion ($P < 0.05$). The increase in Q in response to the second infusion of brady-

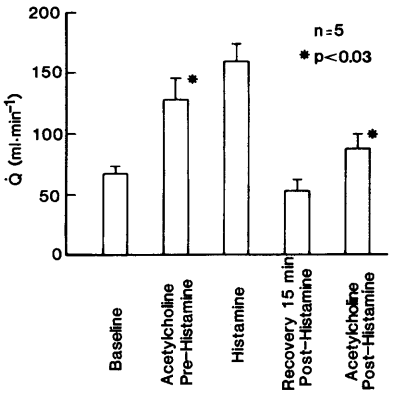

FIG. 5. Q is shown for the baseline period, at the end of 15-min periods of infusion of acetylcholine (1.5 μg · min^{-1}) before and after 2-hr infusion of histamine (150 ng · min^{-1}).

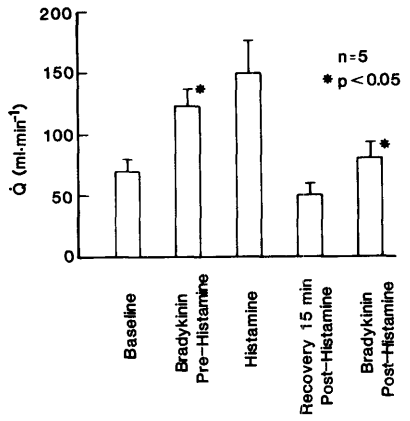

FIG. 6. Q is shown for the baseline period, at the end of 15-min periods of infusion of bradykinin (100 ng·min^{-1}) before and after 2-hr infusion of histamine (150 ng·min^{-1}).

kinin was significantly less than the increase in Q in response to the first infusion of bradykinin ($P < 0.03$) in addition to the significant difference in the absolute level of Q. PAP did not change in these studies and PVR reflected the changes in Q.

Fifteen-minute local infusion of histamine at 150 ng·min^{-1} acetylcholine at 1.5 μg·min^{-1}, or bradykinin at 100 ng·min^{-1} 2 hr apart showed no tachyphylaxis.

B. Crossover studies with 2-hr infusion of bradykinin. Q in response to 15-min infusion of histamine at 75 ng·min^{-1} before 2-hr infusion of bradykinin at 100 ng·min^{-1} was 123 ± 9 ml·min^{-1}, whereas the response after infusion was 93 ± 4 ml·min^{-1} (n = 3).

In one fetus, the response to 15-min infusion of acetylcholine infusion was diminished following 2-hr infusion of bradykinin.

C. Crossover studies with 2-hr infusion of acetylcholine. Q in response to 15-min infusion of histamine at 75 ng·min^{-1} before 2-hr infusion of acetylcholine at 3.0 μg·min^{-1} was 103 ± 6 ml·min^{-1} whereas the response after infusion was 77 ± 5 ml·min^{-1} (n = 3).

In one fetus the response to 15-min infusion of bradykinin was diminished following 2-hr infusion of acetylcholine.

Discussion. In the fetus each lung receives less than 5% of the cardiac output (14). Therefore, infusing a drug directly into the left pulmonary artery allows for study of the drug's action on the pulmonary circulation while minimizing the systemic effects. This is especially important in examining effects over several hours. By use of this method we have found that the pulmonary vasodilation produced by infusion of acetylcholine, histamine, or bradykinin is not sustained over a 2-hr period. Pulmonary blood flow increases to a peak at 10 to 20 min for all three agents but declines steadily toward baseline over the remainder of the 2-hr infusion period. Calculated pulmonary vascular resistance is increased following a 2-hr infusion but not following a 15-min infusion. Repeat infusion with the same or a different drug following a 2-hr infusion resulted in a diminished vasodilatory response.

These findings indicate that mechanisms exist in the fetus that oppose vasodilating stimuli applied to the lung. Our results also demonstrate that fetal pulmonary vascular tone is increased following a 2-hr exposure to the vasodilatory stimuli studied and that vasoreactivity, defined as the response to the same or a different vasodilator, is also altered. In addition, the similarities in the temporal characteristics of the blood flow responses to acetylcholine, histamine, and bradykinin suggest either that some steps in the process of vasodilation are common to all three agents or that there are similarities in the fetal pulmonary vascular response to vasodilation by each of the agents.

Little is known about the temporal response to vasodilating stimuli in the adult lung. In part this is because the adult pulmonary vascular bed is close to being maximally dilated and infusion of vasodilators produces little effect (15). In addition, vasoactive agents which accumulate systemically may affect systemic vascular resistance or change cardiac output, leading to passive changes in pulmonary vascular resistance (15). Pulmonary vasodilation is more easily demonstrated in the adult animal when baseline tone is increased as by means of hypoxia (16, 17). However, in none of these studies have agents been infused for 2 hr. Although time-dependent responses to the vasoconstricting stimulus of hypoxia have been reported (18, 19, 20), the temporal response to pharmacologic pulmonary vasodilators over a 2-hr period, the state of pulmonary vascular tone

after vasodilation, and the effect on vasoreactivity are not known in the adult animal. In the hypoxic newborn lamb, pulmonary vasodilation in response to tolazoline is sustained over a 2-hr period; however, the temporal response to other vasodilators has not been studied (21).

The fetal pulmonary circulation differs in several important aspects from that of the adult or newborn in that pulmonary vascular tone is normally high, the change in flow per increase in pressure is much less (2), and blood flow to the lungs is less than 10% of the combined cardiac output with most right ventricular output shunted through the ductus (14). In the adult lung large changes in flow that occur in response to small changes in pressure make the distinction between active and passive changes in calculated pulmonary vascular resistance difficult. The small changes in flow that occur with changes in pressure in the fetal pulmonary circulation, however, allow interpretation of changes in flow with little or no changes in pressure, as in the current study, in terms of active changes in pulmonary vascular resistance. Because of the high resting tone in the fetal pulmonary circulation, acetylcholine, histamine, and bradykinin were clearly shown to be vasodilators in early studies of fetal pulmonary vasoreactivity (22, 23) in agreement with the adult studies on pulmonary vascular beds with increased tone. More recent studies of fetal pulmonary vasoreactivity have examined the effects of the eicosanoids; however, the temporal characteristics of the responses were not assessed in these studies (24–27).

The low blood flow to the lung in the fetus allows a high concentration of agents to be achieved locally in the lung with little accumulation systemically. Left pulmonary artery infusion of acetylcholine, histamine, or bradykinin resulted in a rapid increase in pulmonary blood flow with little or no effect on pulmonary or systemic pressure. Cessation of infusion through the left pulmonary artery catheter resulted in a rapid falling off in pulmonary blood flow, suggesting that vasodilation in response to left pulmonary artery infusion is primarily a first-pass effect. Right pulmonary artery and main pulmonary artery infusion at the same rates resulted in markedly diminished responses or no response, further confirming the local nature of the effects. In addition, the lack of change in pulmonary artery pressure and aortic pressure, or the gradient between the two suggests that constriction of the ductus was not functionally important in the temporal response to the agents studied.

The results of the present study complement and extend other studies of fetal pulmonary vasoreactivity. The lack of a sustained response of the fetal pulmonary circulation to infusion of acetylcholine has been noted previously but not quantitated (28). We have observed that increases in fetal PO_2 resulted in an increase in pulmonary blood flow followed by a decline toward baseline, also suggesting that mechanisms exist opposing vasodilatory stimuli (5). Pulmonary vasodilation in response to tolazoline in the fetus is not sustained over a 90-min period and the response to a repeat infusion is also markedly diminished (9). Verapamil prolonged the response to tolazoline, suggesting a calcium-dependent mechanism in the loss of responsiveness. The leukotriene end organ antagonists, FPL 55712 and FPL 57231 caused fetal pulmonary vasodilation during a 1-hr infusion and increased pulmonary vascular resistance following infusion (29). Finally, a recent preliminary report suggests that the response to the fetal pulmonary vasodilators PgI_2, PgE_1, PgE_2, and PgD_2 are not sustained over a 2-hr period (30). Our results extend these studies by demonstrating that the fetal pulmonary circulation adapts to several agents with a similar time course, that pulmonary vascular resistance is increased following infusion of these agents, and that the loss of responsiveness to pharmacologic vasodilators is generalized and not restricted to the agent infused for 2 hr.

There are a number of potential mechanisms for loss of responsiveness to the pharmacologic vasodilators we studied. These mechanisms include local effects such as changes in smooth muscle tone or mechanical compression of vessels by increases in perivascular edema, or systemic effects such as changes in blood gas tensions, circulatory reflexes, or in ductal caliber. We found no changes in blood gas tensions, suggesting that the umbilical placental circulatory unit was

not significantly affected by the infusions. Although SAP and PAP did not change with any of the infusions, only acetylcholine resulted in an increase in fetal heart rate, suggesting at least some systemic effect and raising the possibility of reflexes that could also affect the pulmonary circulation. Our studies do not distinguish between the possible local mechanisms of the loss of responsiveness; however, the loss of sensitivity to one agent following exposure to a different agent and the similarity of the temporal responses suggest that the mechanism is not receptor specific.

Current views of vasodilation propose that for a large class of pharmacologic agents, the agonists bind to endothelial cell receptors and then an endothelium-derived relaxant factor (EDRF) is released and causes relaxation of the smooth muscle (31). Acetylcholine, bradykinin, and histamine, at least through H1 effects, are endothelial dependent in several systems (15). Although EDRF has not been identified and although endothelium dependence of vasodilation has not been demonstrated in the sheep, the similarity of the temporal responses to the three pharmacologic vasodilators we studied would be consistent with some change in EDRF release, EDRF activity, or smooth muscle sensitivity to EDRF. Other locally active autocoids released by endothelium might equally as well play a role in modulating the response to pharmacologic vasodilators.

Histamine and bradykinin cause edema in several tissues though the effect of bradykinin in lung is controversial (32). An increase in perivascular edema might cause mechanical compression of vessels and increase calculated pulmonary vascular resistance without active vasoconstriction.

Blood flows and arterial pressures in our studies are similar to those previously reported (14). A significant correlation between peak blood flow achieved with acetylcholine infusion and gestational age has been reported previously (7), though we found a significant correlation with gestational age only with bradykinin infusion. This may be related to our use of the same infusion rate for fetuses of all gestational ages. Since we found that baseline flow was correlated with gestational age, the younger fetuses experienced higher concentrations of agents.

We have found that in the fetal pulmonary circulation vasodilation is not sustained during a 2-hr infusion of acetylcholine, histamine, or bradykinin, and following 2-hr infusion of these vasodilators there is an increase in pulmonary vascular resistance, and a general loss of sensitivity to pharmacologic vasodilators. These findings provide further evidence for the existence of mechanisms that oppose vasodilatory stimuli to the fetal lung. Teleologically, the loss of sensitivity to vasodilating stimuli may serve to shunt blood away from the fetal lung where large flows are unnecessary for gas exchange *in utero* or may serve to protect the lung from deleterious effects of high flows (33). We speculate that persistence of these mechanisms secondary to endogenous production and release of vasodilating agents *in utero* could lead to a lack of responsiveness to vasodilatory stimuli at birth and contribute to the failure of the pulmonary circulation to achieve a normal transition.

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