Bradykinin Production Associated with Oxygenation of the Fetal Lamb

By Michael A. Heymann, M.B., B.Ch., Abraham M. Rudolph, M.D., Alan S. Nies, M.D., and Kenneth L. Melmon, M.D.

ABSTRACT

Previous studies showed that synthetic bradykinin produced in-vitro constriction of the ductus arteriosus of the fetal lamb and guinea pig, constriction of isolated human and lamb umbilical vessels, and pulmonary vasodilatation in the fetal lamb. The present study showed that bradykinin precursor, kininogen, is present in arterial blood of the fetal lamb by 61 days of gestation and that its concentration increases toward term. We studied the bradykinin-generating system in 6 exteriorized fetal lambs. Kininogen concentrations in left atrial blood decreased within 1 to 2 minutes after beginning ventilation with O₂, and free bradykinin was detected in left atrial blood. Pulmonary arterial blood kininogen concentration was not significantly altered. Kininogen concentration in left atrial blood did not fall in 4 other lambs ventilated with N₂ but did fall after subsequent ventilation with O₂. To study the effects of increased oxygenation in utero without lung expansion, 6 pregnant ewes were exposed to hyperbaric O₂ at 3.63 atmospheres absolute. Associated with a mean increase in brachial arterial blood P₀₂ to 44 mm Hg, a value equivalent to that in the ventilated exteriorized fetus, kininogen concentration fell, and free bradykinin was detected in the brachial arterial blood of 3 of the 6 fetuses. In 4 other exteriorized lambs we measured kininogen concentration in brachial arterial and right ventricular blood at frequent intervals up to 30 minutes after ventilation with O₂. The concentration in brachial arterial blood fell before that in right ventricular blood.

The hypothesis is presented that bradykinin is produced from kininogen in the lungs of the fetal lamb when oxygenated, and that the maximal rate of production occurs during the first few minutes after expansion of the lungs or exposure of the ewe and fetus to hyperbaric O₂.

ADDITIONAL KEY WORDS kininogen hyperbaric oxygenation pulmonary blood flow electromagnetic flowmeter
CHEMICAL ASSAYS

Bradykinin—This was measured on 5 ml of whole blood, drawn directly into 10 ml of cold 30% perchloric acid, by a modification of the method of Webster and Gilmore (16). The bradykinin was assayed by the four-point analysis, using contraction of the isolated uterus of an estrus rat. This allows the separation of bradykinin from alkaline amines as previously described (15). A major limitation of this method is that bradykinin may be produced or destroyed in vitro, and care must be taken that these processes are uniform in all samples. To standardize the method, 10 to 100 ng of synthetic bradykinin (Sandoz) was added to a second 5-ml control blood sample from each fetus. The range of absolute recovery of this standard from these control samples varied from 48% to 75% with different animals; on a single sample, duplicate recoveries varied from each other by less than 12%. Because a paired determination of the percent recovery of a known amount of added bradykinin with each sample would have resulted in a significant depletion of blood volume, a single measurement of percent recovery during the control period was used to standardize all samples. All concentrations were then corrected to consider the recovery value as 100% in each animal. The variability of bradykinin concentrations in duplicate analyses of a single sample or in two samples obtained in rapid succession was less than 8% after correction for recovery. The lowest concentration detectable by this method is about 2 ng bradykinin/ml whole blood. Although bradykinin can cause physiologic vascular changes in lower concentrations (14), this does not preclude the useful application of these methods to detect large changes in bradykinin production associated with a physiologic event.

CONFIRMATION OF BRADYKININ BIOASSAY

In view of some of the potential difficulties associated with the variable recovery of bradykinin in the bioassay method as outlined, two fetal lambs were studied to assess the production of bradykinin in a different way. The fact that the fetal lung will respond with pulmonary vasodilatation to extremely small amounts of injected bradykinin (14) was utilized.

With the ewe under spinal analgesia, a fetal lamb at term was exteriorized, with placental flow continuing as previously described (17). Under local anesthesia, through a left thoracotomy incision, a cuff type of electromagnetic flow transducer was placed around the left pulmonary artery. Flow was then measured with a Statham M4000-gated sine-wave electromagnetic flowmeter (17). The system was not calibrated because we were not measuring actual flow rates but only relative changes in flow. Polyvinyl catheters were placed in the femoral and main and left pulmonary arteries. Pressures, heart rate, and flow were recorded on a Beckman Type R Dynograph.

A second pregnant sheep was prepared alongside the above preparation and the fetus exteriorized; using local anesthesia, the trachea was cannulated, taking care that no respiratory effort was made by the lamb and that no air entered the lungs. The tracheal tube was allowed to drain freely into a beaker with the catheter tip under 2 to 6 cm H2O. A fetal carotid artery and maternal femoral artery were cannulated. Blood samples were then obtained from the carotid artery of this fetus for kininogen and bradykinin estimation by the rat uterus bioassay method.
described. Immediately thereafter, a 2-ml carotid arterial blood sample was withdrawn and without delay injected over a 15-second period directly into the left pulmonary artery of the first fetus while pressures and left pulmonary arterial blood flow were being continuously measured. These samples were obtained during a control period, and then at 1-minute intervals after ventilation with 100% O₂.

In both studies, left pulmonary arterial blood flow was unaffected by the injection of carotid arterial blood obtained during the control period. All carotid arterial samples drawn after ventilation produced a transient increase in flow, and baseline flow remained slightly higher after each injection. An equivalent volume of well-oxygenated maternal femoral arterial blood injected over the same time period failed to reproduce this effect, as did the injection of a blood sample obtained from the femoral artery of the fetus in which flow was being measured. No change in femoral or pulmonary arterial pressure occurred with any of the injections. Figure 1 shows the increase in left pulmonary arterial flow following the injection of 2 ml of carotid arterial blood and the effect of 4 ng of synthetic bradykinin injected similarly over 15 seconds for comparison. This amount of bradykinin would give an approximate bradykinin concentration of 0.1 to 0.2 ng/ml blood. The lack of effect of the injection of 2 ml of maternal arterial blood is also demonstrated.

Kininogen.—Blood samples were drawn into polyethylene syringes containing sufficient heparin to make a final concentration of at least 1 unit/ml. The specimens were centrifuged and the plasma removed with siliconized or polyethylene pipettes. Plasma kininogen was measured in duplicate on 0.4 ml of plasma by the method of Diniz and Carvalho (18). Kininogen is expressed as the total amount of bradykinin released from 1 ml of plasma following the addition of trypsin. The values given are therefore not specific measurements of the actual amounts of kininogen present but, rather, indicate the maximal amount of bradykinin that can be produced from substrate present in 1 ml of the sample plasma. Blood kininogen concentrations were measured more frequently because of the small volume of blood required. There are also limitations to this method. Little is known about the kinetics of the breakdown of kininogen, and nonspecific destruction of kininogen can occur without conversion to bradykinin. Therefore an in-vivo decrease in blood kininogen concentration of a specific amount would not necessarily produce an equivalent amount of circulating bradykinin. Nevertheless, in-vivo kininogen depletion in primates (19) and in-vitro depletion in human cord blood (15).
correlates with bradykinin production, especially in those physiologic states in which the release of tryptic enzymes, intravascular thrombosis, and complement activation are unlikely to occur.

**Results**

**BRADYKININ PRODUCTION IN THE FETAL LAMB**

Although the fetal lamb responds to injected synthetic bradykinin (8, 12-14) and fetal blood on contact with glass is capable of producing a bradykinin-like substance (14), there was no knowledge as to the gestational period at which kininogen first appears or of changes in kininogen concentration in the intact fetal lamb during development. To determine this we placed catheters, by methods previously described (17), in the umbilical vessels of 23 fetal lambs with gestational ages of 61 to 148 days and obtained blood samples for kininogen determination from the umbilical artery without further disturbing the fetus. Femoral arterial blood samples were obtained from six ewes for comparison. The kininogen concentration in umbilical arterial blood rose gradually from early gestation to term (Fig. 2). Term fetal and adult kininogen concentrations were similar (mean maternal value 1700 ng/ml plasma). We thus demonstrated that kininogen was present in the blood of the fetal lamb.

**EFFECT OF LUNG EXPANSION WITH OXYGEN ON RELEASE OF BRADYKININ**

Studies were designed to determine whether bradykinin was produced and could contribute to the pulmonary vasodilatation occurring in the fetal lungs during the transition from the fetal to the neonatal circulation.
BRADYKININ PRODUCTION

Six fetal lambs at term were exteriorized with placental circulation continuing, as described before. Through a left thoracotomy, polyvinyl catheters were inserted within purse-string sutures into the left atrium in all six lambs and into the main pulmonary artery in four. The thoracotomy was closed and the fetus allowed a recovery period of 15 to 20 minutes.

Control blood samples for measurement of kininogen were obtained from the left atrium and the pulmonary artery. The fetal lungs were then expanded and ventilated with 100% O₂. Two minutes later, blood samples were again obtained from the same sites. Kininogen concentration in left atrial blood fell from a mean of 1933 (range 950 to 2940) ng/ml plasma to a mean of 739 (range 250 to 955) ng/ml plasma (P<0.01), whereas concentrations in pulmonary arterial blood were essentially unaltered in three of the four lambs in which it was measured (Fig. 3).

Bradykinin concentrations were measured in left atrial blood in only four of the six lambs. No bradykinin was detected in any of these during the control period, but it was present in all within 2 minutes after ventilation (Fig. 4). Left atrial blood PO₂ was measured in four lambs with a Radiometer oxygen electrode and gas analyzer; it rose from an average of 28 (range 23 to 31) mm Hg to an average of 142 (range 38 to 330) mm Hg. At the end of the procedure, the position of the catheters was always confirmed by direct observation.

These studies showed that following positive-pressure ventilation with oxygen, kininogen concentration fell and bradykinin was present in left atrial blood.

ROLE OF OXYGEN VERSUS MECHANICAL EXPANSION IN THE RELEASE OF BRADYKININ

To determine whether the release of bradykinin was effected simply by mechanical expansion of the lungs or by oxygenation of the blood, two series of studies were performed. In the first, four fetal lambs at term were exteriorized and tracheotomies performed, as in the previous group. We decided

---

1Statistical analysis by paired t-test.

Circulation Research, Vol. XXV, November 1969
not to subject the fetus to a thoracotomy and instead inserted a catheter into the brachial artery. As the circulation time from left atrium to brachial artery is only 1 to 2 seconds, a sample from this site would be representative of a well-mixed left atrial sample. A control brachial arterial blood sample was obtained for measurement of kininogen, and in three lambs for Po$_2$ as well. The fetal lungs were then expanded and ventilated with nitrogen. After 2 minutes of ventilation, blood samples were obtained and the ventilating gas was changed to 100% O$_2$. After a further 2-minute period, brachial arterial blood samples were again obtained from measurement of kininogen and Po$_2$.

After ventilation with nitrogen, there was no fall in kininogen concentration in three of the four lambs and no significant fall in the group as a whole ($P > 0.8$) (Fig. 5). After ventilation with oxygen, there was a significant fall in mean kininogen concentration from 2108 (range 1620 to 2600) ng/ml plasma to 1262 (range 950 to 1680) ng/ml plasma ($P < 0.05$).

The average control brachial arterial blood Po$_2$ was 20 mm Hg, and this was not significantly different from the level of 18 mm Hg after ventilation with nitrogen. After ventilation with oxygen, the Po$_2$ rose to an average of 58 mm Hg. These studies thus demonstrated that mechanical expansion alone of the fetal lungs did not result in a decrease in kininogen due to activation of the bradykinin-generating system.

EFFECTS OF HYPERBARIC OXYGENATION

The second series was concerned with the effects of increased fetal oxygenation without exteriorization and without ventilation of the

---

**Figure 6**

Fetal brachial arterial blood kininogen concentrations in 6 lambs when the ewe was breathing O$_2$ at high pressure. Time 0 represents the time when 3.63 ATA was attained. The shaded area represents the period of increased pressure. The time of exposure varied in different animals; the symbol on the shaded area represents the time when decompression began. Control concentrations were obtained at 1 ATA with the mother breathing room air. Recovery samples were obtained 30 minutes after the mother returned to 1 ATA and was breathing room air.

*Circulation Research, Vol. XXV, November 1969*
fetal lungs. Six pregnant ewes with fetal gestational ages ranging from 120 days to term (148 days) were studied in the Duke University Hyperbaric Unit. Following maternal spinal analgesia, the umbilical vein and artery were cannulated. Fetal forelimb and hindlimb veins and arteries were cannulated after delivery of each limb through a small hysterotomy. In three instances, an additional forelimb catheter was advanced into the right ventricle and then into the pulmonary artery. When the position of the catheters was checked after termination of the experiment, one was found to have passed through the ductus arteriosus into the aorta; the other two were in the pulmonary artery. All catheters were secured in place, and the ewe’s abdomen was closed. More detailed descriptions of these methods have been published previously (17). Following a recovery period of 30 minutes, a control sample for measurement of blood bradykinin and \( \text{Po}_2 \) and duplicate samples (three in one fetus) for kininogen were obtained from the brachial artery. Kininogen concentration in pulmonary arterial blood was also measured in two lambs with pulmonary arterial catheters. The ewe was then given 100% \( \text{O}_2 \) to breathe, using an oxygen hood. The ewe and fetus were exposed to pressure (39 psi) to reach 3.63 atmospheres absolute (ATA). The time taken to reach maximal pressure varied from 4 to 16 minutes, with an average of 7.7 minutes, and the time taken to obtain at least three complete sets of blood samples while at 3.63 ATA was 15 to 28 minutes. The individual durations of maintenance at 3.63 ATA for each animal are shown on the shaded area in Figure 6.

Changes in brachial arterial blood kininogen concentrations are plotted in Figure 6. There was a decrease in each of the six lambs; the lowest level occurred in 5 within the first 7 minutes. Comparison by t-test of the mean of the five paired controls and the mean of the paired samples obtained between 6 and 15
minutes after reaching 3.63 ATA showed a significant fall in kininogen concentration ($P < 0.01$). After the initial decrease in brachial arterial blood kininogen, concentrations in two of the lambs showed some spontaneous recovery toward the control values, but in the others leveled off with little further change. In the two lambs in which pulmonary arterial blood kininogen concentrations were measured, these decreased as well, paralleling the brachial arterial concentrations but at a slightly higher level.

Bradykinin was not detected in the control period in any of the lambs. In three, bradykinin was present after oxygenation; the concentrations are shown in Figure 7. In the other three, none was detected during the period of hyperbaric oxygenation. Brachial arterial blood $P_{O_2}$ values rose from an average of 19 mm Hg during the control period to an average of 44 mm Hg during hyperbaric oxygenation.

The ewe was then allowed to breathe room air and the hyperbaric period terminated for each animal (Fig. 6). The time required for decompression varied according to the time spent at maximal pressure. After again reaching 1 ATA, a 30-minute recovery period was allowed, and brachial arterial blood samples were obtained in five lambs for measurement of kininogen concentrations. These were not significantly different from the means of the control values ($P > 0.8$).

Bradykinin was measured in only one lamb during recovery. In this lamb (Figs. 6 and 7, solid circles) the kininogen concentration was lower during the recovery period than at the termination of the hyperbaric period. Bradykinin concentration was elevated when kininogen was falling and fell as kininogen concentration rose. This indicated active bradykinin production with kininogen depletion at the same time.

Brachial arterial blood $P_{O_2}$ values during the recovery phase were not significantly different from those obtained during the control period ($P > 0.8$). Thus an increase in fetal arterial $P_{O_2}$ alone, without mechanical expansion and ventilation of the lungs, was associated with the production of bradykinin (probably in the lungs) in three of six fetal lambs, with a resultant depletion of kininogen. Unlike three of the four animals in the earlier group of experiments, pulmonary arterial blood kininogen concentrations did fall, although not to the same degree as in brachial arterial blood.

**PULMONARY BLOOD FLOW DURING HYPERBARIC OXYGENATION WITH BRADYKININ PRODUCTION**

In three of the six fetal lambs studied during hyperbaric oxygenation, umbilical blood flow was measured by the antipyrine method (17), and cardiac output and distribution of flow to organs were measured using injections of nuclide-labeled microspheres (20). Flows and distribution were measured during the control period and then immediately before the end of the period of hyperbaric oxygenation in each animal. Umbilical blood flow and cardiac output showed no marked or consistent changes and pulmonary and systemic arterial blood pressures were unaltered. Pulmonary blood flow showed a considerable rise with hyperbaric oxygenation. Expressed as a percent of cardiac output, pulmonary flow in the individual lambs increased from 2.3% to 5.4%, 5.6% to 32.5%, and 3.8% to 8.2%. These increases are less marked than at the time of birth but the presence of an undisturbed, low-resistance placental circulation is possibly responsible for this disparity, since in the fetus changes in pulmonary blood flow depend on the relative resistances between the pulmonary and systemic vascular beds. Changes in kininogen and bradykinin concentrations in these three lambs are shown in Table 1.

**TIME FACTORS INVOLVED IN THE RELEASE OF BRADYKININ**

Pulmonary arterial blood kininogen concentrations fell in the fetuses studied during hyperbaric oxygenation but not in three of the four exteriorized ventilated lambs. Since the former group was followed for a longer time, we wondered whether this change in pulmonary arterial kininogen concentration was time related. This led to a group of experiments in which we examined more closely the time sequence of kininogen concentration changes.
in both the brachial and pulmonary arterial blood. Four fetal lambs at term were exteriorized and the trachea cannulated as described. In these animals small doses of succinylcholine (0.25 mg/kg/min iv) were administered to the fetus to prevent movement and facilitate the procedure. A brachial artery was cannulated and a catheter passed from a peripheral forelimb vein into the right ventricle. Once again, catheter position was checked carefully at the end of each experiment. Two or three control samples were obtained from both the brachial artery and right ventricle to measure kininogen concentrations and from the brachial artery to measure Po2. The lungs were then ventilated with 100% O2, and samples for kininogen from both sites obtained every minute for 5 minutes, then every 2 minutes for an additional 6 minutes, and at varying times thereafter for a maximum of 30 minutes. Brachial arterial Po2 was measured as well. In all instances there was no significant difference between the right ventricular and brachial arterial kininogen concentrations during the control period (P = 0.8). After ventilation, the right ventricular blood concentration remained consistently higher than the brachial arterial concentration. An example of one of these studies is shown in Figure 8. Brachial arterial Po2 rose from an average of 23 mm Hg to an average of 160 mm Hg.

CONFIRMATION OF BRADYKININ BIOASSAY: BRADYKININ PRODUCTION IN THE VENTILATED FETUS

Carotid arterial blood kininogen concentration after ventilation in both animals followed patterns similar to those described earlier. Bradykinin concentrations in carotid blood rose immediately in one fetus to peak at a concentration of 64 ng/ml whole blood 2 minutes after ventilation. In the second fetus, the appearance of bradykinin in carotid arterial blood was delayed till 6 minutes after ventilation, and the peak concentration a

---

**TABLE 1**

<table>
<thead>
<tr>
<th>Fetus</th>
<th>Time* (min)</th>
<th>Kininogen (ng/ml plasma)</th>
<th>Bradykinin (ng/ml blood)</th>
<th>Cardiac output to lungs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>1200</td>
<td>0</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1450</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>1350</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>720</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>950</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>1000</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>1475</td>
<td>0</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1500</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>975</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>530</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>500</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>1070</td>
<td>0</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1000</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1170</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>575</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>600</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>675</td>
<td>8.2</td>
<td></td>
</tr>
</tbody>
</table>

*Time in minutes after reaching 3.63 ATA.
minute later was 3.5 ng/ml whole blood. In this fetus at the time of the last sample 10 minutes after ventilation, no bradykinin was detectable by the estrus rat uterus bioassay. The sample obtained immediately afterwards and injected into the left pulmonary artery of the second fetus produced the change shown in Figure 1.

These studies showed that following ventilation with oxygen, some substance was released that caused pulmonary vasodilatation. The effect was not due to the injection of blood with a high PO₂ since maternal arterial blood failed to produce an effect. Although this method could not establish the exact identity of the substance, small amounts were

### TABLE 2

Simultaneous Umbilical Arterial and Umbilical Venous Blood Kininogen Concentrations in Four Lambs before Lung Expansion and after Expansion with 100% O₂ and in Two Animals before and during Hyperbaric Oxygenation

<table>
<thead>
<tr>
<th>Arterial</th>
<th>Venous</th>
<th>Arterial</th>
<th>Venous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before Expansion</td>
<td>1850</td>
<td>1800</td>
<td>620</td>
</tr>
<tr>
<td>1880</td>
<td>1950</td>
<td>1120</td>
<td>1280</td>
</tr>
<tr>
<td>2750</td>
<td>2500</td>
<td>1160</td>
<td>1750</td>
</tr>
<tr>
<td>1850</td>
<td>2000</td>
<td>1300</td>
<td>1200</td>
</tr>
<tr>
<td>Before Hyperbaric Oxygenation</td>
<td>985</td>
<td>1075</td>
<td>575</td>
</tr>
<tr>
<td>1000</td>
<td>1070</td>
<td>600</td>
<td>670</td>
</tr>
</tbody>
</table>

Values are ng/ml plasma.
detectable, and as the response was similar to that produced by synthetic bradykinin, possibly it was bradykinin. In addition, increased bradykinin concentrations were detected in some samples by the rat uterus bioassay method; vasodilatation occurred in response to the injection of samples obtained at the same time.

Discussion

The exact mechanism producing pulmonary vasodilatation after birth is not yet fully defined. Colebatch et al. (1) showed that gaseous expansion of fetal lungs with a mixture of 3% O₂ and 7% CO₂ in N₂, while not significantly altering arterial pH and blood gases, produced pulmonary vasodilatation. However, further pulmonary vascular dilatation results from a decrease in CO₂ content or increase in O₂ content of the ventilating gas (3). Expansion of the lungs with deoxygenated dextran or saline solution produced little change in pulmonary vascular resistance, but when oxygenated dextran solution or arterial blood was used, pulmonary vascular resistance fell markedly (2). These responses must be locally effected, since they are not prevented by vagotomy and sympathetic blockade. Dawes (21) has suggested that at least one-third of the total fall in resistance is due to the gaseous expansion alone, and the remainder is then produced by the increase in PO₂ and fall in PCO₂. However, the exact mechanisms by which the increase in PO₂ actually mediates these changes in pulmonary vascular resistance are unknown.

We have shown that kininogen is present in the blood of the fetal lamb as early as 61 days of gestation and that with maturation of the fetus the concentration increases. We were able to demonstrate that the bradykinin-generating system was activated when fetal arterial PO₂ was increased, either by ventilating the fetal lungs with oxygen or by exposing the ewe and fetus to hyperbaric oxygenation without inflating the lungs. The evidence we have obtained suggests that this release of bradykinin occurred in the lungs. We would have preferred to obtain pulmonary venous blood samples to assess the release more accurately; however, this was precluded by the extensive surgical manipulation required. We fully appreciate that left atrial blood samples may not represent true pulmonary venous blood, since during fetal life the left atrium receives a large volume of blood shunted across the foramen ovale from the inferior vena cava, and this in turn comprises largely umbilical venous blood. Expansion of the lungs results in an increased left atrial pressure due to increased pulmonary venous return with a resultant marked decrease or even complete cessation of right-to-left shunt across the foramen ovale. Therefore, after ventilation, a left atrial blood sample would in fact represent predominantly pulmonary venous blood. This would also be true in the animals subjected to hyperbaric oxygenation, since with an increase in PO₂ of blood perfusing the lungs, pulmonary vascular resistance falls, with expected similar effects on foramen ovale shunting. To completely exclude the possibility that changes in concentration of kininogen in left atrial blood could have been produced by changes in that in the umbilical vein, umbilical venous and arterial blood samples were obtained before and after ventilation of the fetal lungs with oxygen in four lambs, and before and during hyperbaric oxygenation in two lambs (Table 2). There were no significant differences between the umbilical venous and arterial concentrations in either the control period or following oxygenation (P > 0.08). These studies suggested, therefore, that the decreases in kininogen concentration in left atrial blood were in fact a true reflection of changes in pulmonary venous concentrations. However, the remote possibility that kininogen depletion occurred in organs of the lower body of the fetus was not excluded.

Bradykinin could not be detected at all in left atrial blood in three instances, or was detected only well after the maximal fall in kininogen. (Table 1). This does not exclude the fact that it was released in the lung. Bradykinin is effective in extremely small doses, and in some experiments (14) it has
been shown that injection of 1 to 2 ng of bradykinin directly into the pulmonary artery produces pulmonary vasodilatation. Blood concentrations following the injection of this amount may not be readily detected by the assay methods we used. Recent studies by Ferreira and Vane (22) have shown that in adult cats, up to 80% of intravenously injected bradykinin may be taken up by the lungs or destroyed in a single passage through the lungs. This, too, and the possibility that the bradykinin produced was actually utilized in the lungs to produce pulmonary vasodilatation could explain the apparent disparity between the amount of kininogen depletion and the small and variable amounts of bradykinin detected in left atrial blood.

The bradykinin concentrations in arterial blood in several of the fetal lambs were in the same range as those considered by Nies et al. (19) to play a role in the cardiovascular collapse associated with endotoxic shock in monkeys. There was no effect of these high bradykinin concentrations on cardiac output, umbilical blood flow, systemic arterial blood pressure, and heart rate in the fetal lambs. Infusion for 15 seconds into the descending aorta of a fetal lamb of sufficient synthetic bradykinin (2 μg) to produce a concentration approximately equivalent to the highest brachial arterial concentration obtained induced no change in systemic arterial pressure. Two- and threefold amounts produced a minimal fall in pressure (2 to 5 mm Hg). The injection of 100 μg of synthetic bradykinin directly into the umbilical artery had no effect on systemic arterial pressure. Nies et al. (19) have suggested that other mechanisms such as endotoxic blockade of sympathetic reflexes, rather than an increased bradykinin concentration alone, could be responsible for the sustained peripheral vascular dilatation in endotoxic shock.

In the present studies, the only physiologic change required to initiate bradykinin production was an increase in P0₂. Mechanical expansion of the lungs in the absence of oxygen could not be demonstrated to play any role in bradykinin production. Following the exposure to oxygen, kininogen was rapidly depleted, with a considerable and rapid fall in kininogen concentration in left atrial blood. Our initial studies did not adequately show the course of these events; however, the latter two groups of studies demonstrated that the kininogen concentration of blood perfusing the lungs remained at control levels for 2 to 3 minutes, and only then started to fall. The fall in pulmonary arterial kininogen concentration in the one fetal lamb in Figure 3 could possibly be explained by the fact that the rate of fall of pulmonary arterial kininogen may be variable or that the time taken to oxygenate the fetus may also be variable.

These observations indicated that sudden bradykinin production occurred in the fetal lungs and was dependent only on an increase in P0₂ regardless of whether this was produced by ventilation with oxygen or by hyperbaric oxygenation. From these studies, we were unable to assess the level of P0₂ required to initiate the process, but in-vitro studies (15) suggested that a P0₂ of more than 35 mm Hg was the critical level. The duration and amount of total bradykinin production could not be accurately assessed. Although the initial rapid decrease in kininogen suggests that bradykinin was rapidly produced and that the rate of bradykinin generation then decreased, we do not know what mechanisms regulate kininogen production at this time of rapid turnover. There is some evidence in the rhesus monkey (19) that kininogen may be produced and released more rapidly than would be expected of most alpha-2-globulins, and this may also be true in the lamb. Also, the level of bradykinin in left atrial blood may not necessarily reflect the actual amount produced, since it may be utilized in varying amounts and rates in the lung.

We have not determined the direct mechanism of bradykinin production in the lamb. In the human, granulocyte kallikrein or kallikrein activator has been implicated in bradykinin production (15). We measured differential granulocyte counts in the left atrial blood in some of the lambs in this study but found no
direct relation between granulocyte count and bradykinin production. It is also clear that a temperature fall could not have been a major stimulus for bradykinin production in these studies, since in the hyperbaric studies the fetal lamb remained in utero.

This study has shown that in the lamb bradykinin is produced in sufficient quantities to produce pulmonary vasodilatation; bradykinin production is oxygen dependent and not initiated by mechanical expansion of the lung; it is produced in the lung but may possibly be formed in the systemic circulation also, and the production is associated with pulmonary vasodilatation occurring with the onset of respiration in the lamb. However, we have not as yet been able to determine whether the effects of oxygen on the pulmonary circulation are completely mediated through local bradykinin release, or whether oxygen has an independent direct vasodilator effect as well.

Acknowledgment

The authors wish to express their gratitude to Mr. Sidney Steinberg for his advice on instrumentation, and to Misses Alice Lytle, Michele Sanda, Dorothy Reese and Christine Mueller and Mr. Robert Sirbu for their skillful technical assistance.

The hyperbaric studies were performed at Duke University Medical Center, Durham, North Carolina. We are deeply grateful to Drs. Saltzman, Brumley and Risemberg and the members of the hyperbaric unit for affording us the opportunity to perform these studies and for their invaluable assistance during them.

References


21. **Dawes, G. S.** Foetal and Neonatal Physiology.

Bradykinin Production Associated with Oxygenation of the Fetal Lamb
MICHAEL A. HEYMANN, ABRAHAM M. RUDOLPH, ALAN S. NIES and KENNETH L. MELMON

Circ Res. 1969;25:521-534
doi: 10.1161/01.RES.25.5.521

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1969 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/25/5/521

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/