

Inactivation of Bradykinin in the Pulmonary Vascular Bed of Newborn and Fetal Lambs

By Beat Friedli, Geraldine Kent, and Peter M. Olley

ABSTRACT

Bradykinin is largely inactivated in one passage through the pulmonary vascular bed of adult animals. We studied pulmonary inactivation of bradykinin in newborn lambs, fetal lambs at term, fetal lambs preterm, and adult ewes by comparing the systemic pressure response to infusions proximal to the pulmonary vascular bed with the response to infusions distal to the pulmonary vascular bed. A high degree of inactivation was confirmed in mature ewes ($93 \pm 1.2\%$); newborn lambs showed significantly less pulmonary inactivation ($68 \pm 5.2\%$) and fetal lambs at term showed only $46 \pm 4.8\%$ inactivation. In the preterm fetuses (gestational age 110–128 days), no inactivation could be demonstrated in the pulmonary vascular bed. The return to base-line diastolic pressure after the infusion was discontinued was much slower in the fetus than it was in the newborn and the adult animal, indicating a prolonged effect of bradykinin. The results suggest that inactivation of bradykinin is a pulmonary function that develops late in fetal life and is still less than adult levels at birth. This finding could be of particular interest if bradykinin participates in mediating the circulatory changes occurring at birth.

KEY WORDS vasoactive substances circulatory changes at birth
systemic pressure response technique lung bradykininase hypotensive peptides
ewes fetal circulation

■ Vasoactive substances may be metabolized in the adult pulmonary circulation. Vane (1) and Ferreira and Vane (2) have demonstrated pulmonary activation of angiotensin I, inactivation of bradykinin, serotonin, and prostaglandin E, and free passage of angiotensin II. Using the blood-bathed organ technique, Ferreira and Vane (2) found an average of 78% extraction for bradykinin in cat lungs. Biron (3) and Scholz and Biron (4) used the systemic pressure response technique in rats and dogs and found pulmonary inactivation of bradykinin between 75% and 90%. Stewart (5) reported bradykinin pulmonary inactivation rates of up to 98%.

Bradykinin may be a mediator in the circulatory adjustments to birth (6, 7); it dilates the fetal pulmonary vasculature (8, 9), constricts the umbilical artery in vitro (10, 11), and causes contraction of the ductus arteriosus in vitro (12). Bradykinin

production is stimulated by raising the arterial oxygen tension of fetal lambs (13).

If bradykinin participates in the circulatory changes at birth, it might be more persistent in the fetal and the neonatal circulations than it is in the adult circulation. Therefore, we determined bradykinin inactivation in the fetal and the neonatal pulmonary circulation of the lamb, using the systemic pressure response technique.

Methods

Bradykinin inactivation was studied in five adult ewes, five newborn lambs, and eight fetal lambs with intact placental circulation by the systemic pressure response technique.

Ewes were anesthetized with sodium pentobarbital (15 mg/kg, iv), intubated, and ventilated on an Air Shields ventilator with a mixture of 30–50% O₂ in N₂O to which small amounts of methoxyflurane were added. A venous catheter was introduced through the left jugular vein and advanced to the right atrium to permit infusion proximal to the lung. Another catheter was advanced through the left carotid artery into the left ventricle for infusion distal to the lung. A third catheter was inserted into the carotid artery to record blood pressure.

Newborn lambs, ranging in age from 6 hours to 30 days, were anesthetized and ventilated as described for the ewes. The ductus arteriosus was ligated through a left thoracotomy, and the prepulmonary catheter was

From the Department of Cardiology and Research Institute, The Hospital for Sick Children, Toronto M5C 1X8, Canada.

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Please send reprint requests to Dr. P. M. Olley, Hospital for Sick Children, Toronto M5C 1X8, Canada.

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inserted directly into the pulmonary artery through a purse-string suture. Thus, shunting of infused material through the ductus arteriosus or the foramen ovale was avoided. The postpulmonary catheter was inserted from the left carotid artery into the ascending aorta until the resistance of the aortic valve was encountered. The correct position of the catheter was checked at postmortem examination. A third catheter to measure blood pressure was advanced from the femoral artery to the thoracic aorta. Phasic pressure was measured with a Statham P23D transducer and was recorded on a Brush direct-writing recorder.

Fetal lambs were exteriorized through a small incision in the uterine wall after a laparotomy was performed on the pregnant ewe near term. Four fetuses were actually term (gestational age 141–148 days), and four others were premature (gestational age 110–128 days). The head of the exteriorized fetus was covered with a surgical glove to avoid ventilation. The uterine wall was fixed to the abdominal skin of the fetus to protect the umbilical cord. Catheters were inserted as described for the newborn lambs. Ligation of the ductus arteriosus in fetuses was regularly followed by a small drop in systemic blood pressure, which stabilized rapidly and was well tolerated for several hours.

Pressure Assay.—Synthetic bradykinin¹ was diluted

¹Courtesy of Sandoz Pharmaceuticals Ltd.

to obtain solutions of 0.2 $\mu\text{g}/\text{ml}$ for fetal and neonatal experiments. In ewes, solutions of 0.5 $\mu\text{g}/\text{ml}$ were used for infusion distal to the lung and of 2 $\mu\text{g}/\text{ml}$ for infusion proximal to the lung. The assay was started once a stable blood pressure had been obtained. The infusions were continued for 2 minutes using a Harvard constant-infusion pump at rates of 2–15 ml/min. The volume infused never exceeded 0.5% of the animal's body weight in any single infusion. The bradykinin-induced fall in blood pressure reached a plateau within 2 minutes in every infusion.

Graded doses of bradykinin were infused alternately through the prepulmonary and the postpulmonary catheter, to obtain dose-response curves for each route of administration. In two animals, three different doses were given to demonstrate the linear relationship between the logarithm of the dose and the pressure response. In all other animals, a low dose and a high dose were given alternately through the prepulmonary and the postpulmonary route; each infusion was then repeated once in reverse order. This design minimized the effect of tachyphylaxis during the study.

Calculation of Inactivation.—Plotting the pressure response against the logarithm of the dose for each route results in two parallel dose-response curves. By interpolation, the dose required to decrease diastolic blood pressure by one-third of its base-line value was

TABLE 1

Doses Required to Achieve a One-Third Drop in Diastolic Pressure for Prepulmonary and Postpulmonary Infusion and Percent Inactivation

Animal	Wt (kg)	Age or gestation (days)	Postpulmonary dose ($\mu\text{g}/\text{kg min}^{-1}$)	Prepulmonary dose ($\mu\text{g}/\text{kg min}^{-1}$)	Ratio	% Inactivation
Ewe						
1	83		17	485	28.5	96
2	68		18.5	320	16.5	95
3	62		24	255	10.6	91
4	61		22	400	18.1	95
5	91		41	425	10.4	90
MEAN \pm SE	73.0 \pm 5.97		24.5 \pm 4.40	377.0 \pm 40.39	16.8 \pm 3.30	93.4 \pm 1.20
Newborn lamb						
1	4.2	0.25	78	380	4.9	79
2	6.6	6	76	200	2.6	62
3	9.6	14	62	164	2.6	62
4	6.7	10	65	362	5.6	82
5	12.6	30	63	140	2.2	55
MEAN \pm SE	7.94 \pm 1.445		68.8 \pm 3.39	249.2 \pm 50.71	3.58 \pm 1.553	68.0 \pm 5.28
Fetal lambs at term						
1	3.8	142	590	980	1.6	40
2	4.1	141	116	225	1.9	49
3	4.8	148	93	150	1.6	38
4	4.6	145	59	145	2.4	59
MEAN \pm SE	4.32 \pm 0.228	144.0 \pm 1.52	214.5 \pm 125.71	375.0 \pm 202.49	1.87 \pm 0.189	46.5 \pm 4.8
Premature fetal lambs						
1	2.7	122	300	300	1.0	0
2	3.2	128	340	340	1.0	0
3	2.0	111	540	540	1.0	0
4	2.2	110	620	620	1.0	0
MEAN \pm SE	2.52 \pm 0.181	117.7 \pm 4.36	450.0 \pm 77.24	450.0 \pm 77.24	1.0	0

determined for the prepulmonary and the postpulmonary route. From the ratio of these doses, inactivation was calculated by the formula

$$100 \frac{\text{postpulmonary dose}}{\text{prepulmonary dose}} \times 100 = \% \text{ inactivation. (1)}$$

Statistical Methods.—Analysis of variance was carried out to relate inactivation in the four groups with three orthogonal comparisons. Student's *t*-test was used to compare the pressure responses to prepulmonary and postpulmonary injections.

Results

Table 1 shows the doses required to decrease diastolic blood pressure by one-third of its base-line value by each route of administration for each group; the age and the weight of the animals are also given. Figure 1 is a graphic representation; individual percents of inactivation are given as well as the mean \pm SE in each group. In ewes, the dose of bradykinin infused proximal to the lungs had to be ten or more times greater than the dose infused into the systemic vascular bed to achieve a similar pressure response, indicating 90–96% inactivation in the lung (Fig. 2A). The pressure curve typically showed a rapid drop toward a minimum and then a slight increase to a plateau. This characteristic shape was seen for both prepulmonary and postpulmonary infusions. Pressure responses tended to be slightly smaller for a given dose in some ewes during the second run due to tachyphylaxis. After discontinuing the infusion, the diastolic pressure returned to the base-line value. In newborn lambs (Fig. 2B) the prepulmonary dose required to produce the same pressure drop had to be about two to five times greater than the postpulmonary infusion, indicating 55–82% inactivation. In fetal

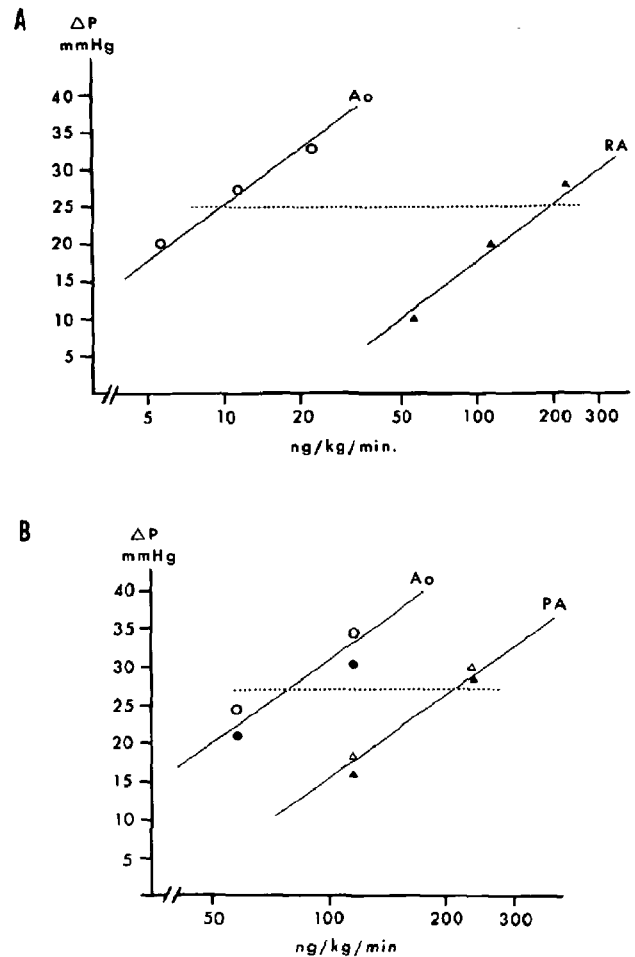


FIGURE 2

A: Dose-response curves to infusions of bradykinin into the aorta (Ao) and right atrium (RA) in mature ewe no. 2. Pressure response (ΔP) is a linear function of the logarithm of the dose. The broken line is drawn at a ΔP of one-third of the diastolic base-line pressure; from the point where it crosses the dose-response curves, the dose needed to induce this pressure drop can be determined for either route of infusion. **B:** Dose-response curves to bradykinin in newborn lamb no. 2. The curves are closer together than those in A, indicating less inactivation. Infusions were repeated once. The response to the second run (solid symbols) was slightly less than the response to the first run (open symbols), suggesting some degree of tachyphylaxis.

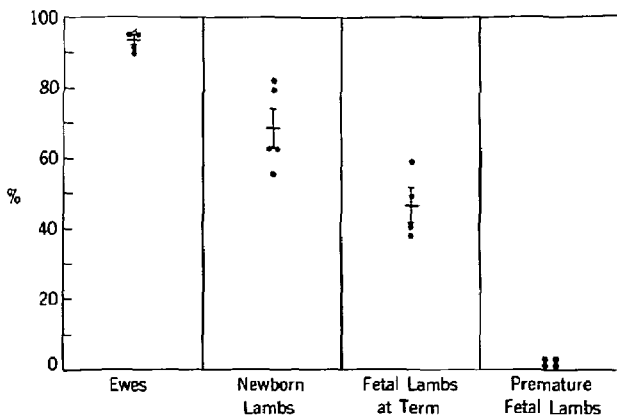
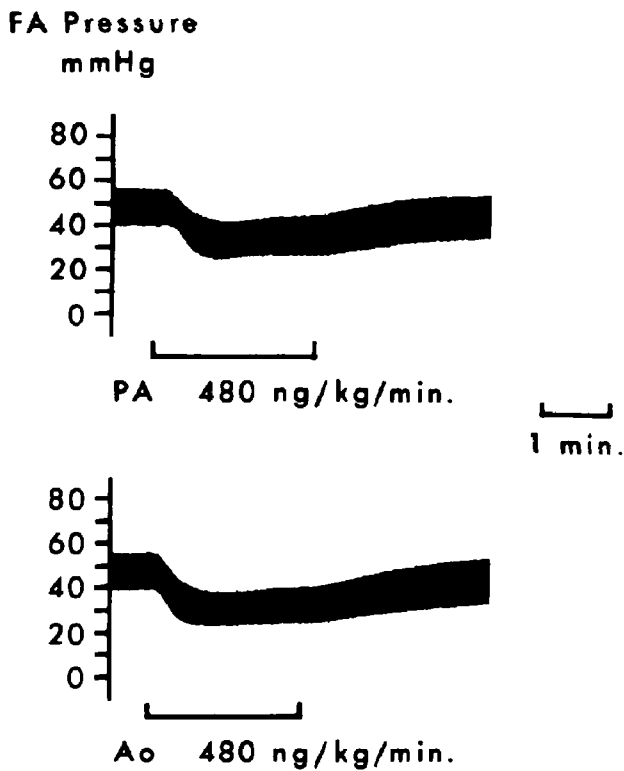


FIGURE 1

Graph showing pulmonary inactivation in percent for the four groups. Individual results and means \pm SE are given.

lambs, inactivation rates were even lower. The mean was $46 \pm 4.8\%$ in the four fetuses at term. In none of the four premature lambs could any inactivation of bradykinin be demonstrated, i.e., the same dose infused proximally and distally to the lung gave the same pressure response (Fig. 3). Statistically significant differences ($P < 0.01$) were found for all comparisons: ewes vs. newborns,



BRADYKININ INFUSIONS

FIGURE 3

Examples of pressure responses in fetal lamb no. 5 3 weeks before term recorded in the femoral artery (FA). The same dose resulted in the same pressure response whether the infusion was made into the pulmonary artery (PA) or the aorta (Ao).

newborns vs. term fetuses, and term fetuses vs. preterm fetuses.

Considering the pressure response to post pulmonary infusions on a mg/kg basis, it appears that the ewes required the lowest dose to induce a pressure fall of one-third of the base-line value. Newborn lambs required a slightly higher dose, and fetuses required a markedly higher one.

The pressure response lasted longer in fetuses, as demonstrated by the slower return to base line after the infusion had been stopped. To substantiate this difference, we measured the half-life of the pressure response, i.e., the time from the moment the infusion was discontinued to the time when the diastolic pressure had returned halfway to base line. Figure 4 shows the half-life in each group as seen after a standard pressure response of one-third of the base-line diastolic pressure. The pressure response is short-lived in ewes (31 ± 11.3 sec), slightly longer in newborn lambs (42 ± 15.1 sec-

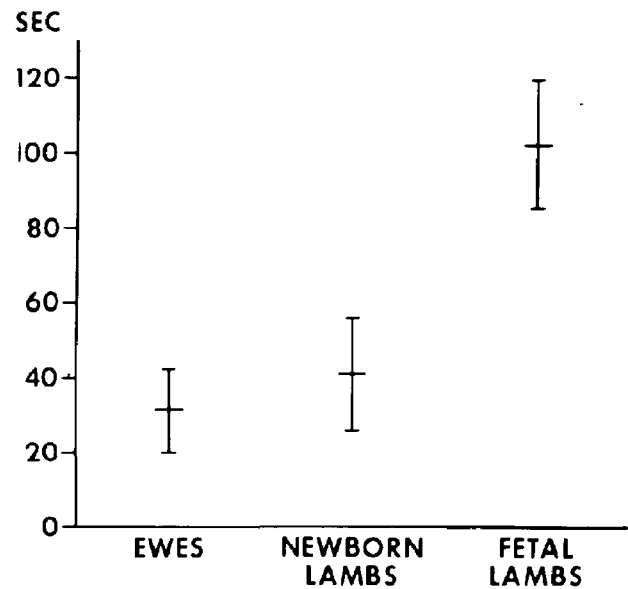


FIGURE 4

The half-lives of the pressure responses in seconds, means \pm SE, are shown for each group. Half-life is defined as the time elapsed from the moment the infusion was discontinued to the moment the diastolic pressure had returned halfway to base-line value. This time was much longer in the fetus than it was in the newborn or the mature ewe.

onds), but markedly longer in fetuses (103 ± 17.3 seconds).

The response of the pulmonary artery pressure to the infusions was recorded in two fetuses by an additional Teflon cannula inserted directly into the pulmonary artery. A drop in pulmonary artery pressure followed by a stable plateau was found after both aortic and pulmonary artery infusion; the response to the aortic infusion was only 15–20% less than the one obtained by pulmonary artery infusion.

Blood gases were monitored during six fetal experiments: pH averaged 7.34 ± 0.036 at the beginning and 7.27 ± 0.030 at the end of the pressure test. This difference was not significant. The P_{O_2} was 19.2 ± 2.77 mm Hg at the beginning and 18.0 ± 1.44 mm Hg at the end of the experiment. The term fetuses had a slightly lower P_{O_2} (14.5 ± 0.86 mm Hg) than did the preterm fetuses (21 ± 1.95 mm Hg), but this difference was not statistically significant. Blood gases in newborn lambs and adult ewes were not routinely monitored; however, in three ewes the P_{O_2} varied from 80 to 110 mm Hg.

Discussion

The systemic pressure response assay has been used extensively in recent years to study the

metabolism of vasoactive substances in various organs (3, 4, 14, 15). The assay compares the pressure responses to infusions given proximal and distal to the organ under study, using the animal's systemic vascular bed as the target organ. To study the fate of vasoactive hormones in the lung, infusions into the pulmonary artery and the pulmonary veins or left atrium would be ideal. Because the left atrium is not readily accessible and because of the rapid transit time through the left heart, using the left ventricle or the ascending aorta just above the aortic valves as the postpulmonary site seems fully justified. For the prepulmonary infusion, we chose the right atrium in ewes but used the pulmonary artery in lambs and fetuses to avoid shunting of bradykinin to the left heart through a patent foramen ovale. This slightly different approach, pulmonary artery vs. right atrium, is unlikely to influence the results, because the transit time from the right atrium to the pulmonary artery is very short. To make sure, we catheterized the pulmonary artery in two ewes and compared the response to infusions in the pulmonary artery and right atrium. The same response was obtained.

The fetal circulation presents several problems with regard to the application of the systemic pressure response technique. First, the pulmonary and the systemic circulations are parallel rather than in series (7). It is, therefore, essential to tie the ductus arteriosus. This procedure was tolerated well by most fetuses for the duration of the experiment. The systemic pressure stabilized rapidly on a slightly lower level after ligation.

The umbilicoplacental circulation is another factor that could potentially interfere with the systemic pressure response. It has been shown in vitro that human and sheep umbilical arteries may constrict when they are exposed to bradykinin (10, 11), although this phenomenon is not invariably the case (16). In fetal lambs in vivo, however, constriction of umbilical arteries in response to infusions of bradykinin could not be demonstrated by Assali et al. (9). Pressure and flow both decreased, leaving resistance unchanged, and these changes were interpreted as passive alterations in response to the general drop in systemic blood pressure. Umbilicoplacental flow remained adequate during bradykinin infusion to preserve normal fetal PO_2 and pH in both the study by Assali et al. (9) and this present study.

The fetal pulmonary vascular bed is exquisitely sensitive to bradykinin and responds regularly with a marked vasodilation. In the neonatal and adult

pulmonary circulation with its low resistance, only small, variable responses to bradykinin have been observed (17). If the drop in pulmonary vascular resistance were markedly different in response to infusions into the pulmonary artery and the aorta, the choice of infusion site could influence the systemic pressure response. We measured pulmonary artery pressure in two fetal lambs by an additional catheter in the left pulmonary artery. A drop in pulmonary artery pressure occurred in response to infusions into both the aorta and the pulmonary artery. This decrease was only about 15–20% less after aortic infusion than it was after pulmonary artery infusion. Therefore, this procedure is unlikely to influence the systemic pressure response. We believe, therefore, that the systemic pressure response technique is valid in fetal lambs with intact placental circulation.

Almost complete inactivation of bradykinin in one passage through the pulmonary vascular bed of adult animals has been demonstrated for various species (2–5). Inactivation of vasoactive hormones in the perinatal period has been studied previously by Hebert et al. (15) with a slightly different technique (bolus injections). Their results for bradykinin inactivation in newborn lambs are similar to ours. They did not, however, find a significant difference between the inactivation in newborn lambs and that in adult ewes, which seems to be due to the fact that their control ewes had surprisingly low inactivation rates, i.e., rates lower than those found in various adult animal species in a number of studies. Bradykinin inactivation in premature fetuses has not previously been adequately investigated. It appears from our study using the systemic pressure response technique that pulmonary inactivation is an age-related process in lambs. It is undetectable in the preterm fetus, low in the term fetus, and significantly higher in the newborn lamb, but still well below adult levels. Ventilation of the lungs and oxygenation of the blood could be significant factors in the difference between the term fetus and the newborn. However, considering all groups, no clear correlation exists between arterial PO_2 and inactivation. Term fetuses with a higher inactivation tended to have a lower PO_2 than the premature fetuses (although this difference was not statistically significant). The rise in PO_2 postnatally may partly explain the difference between prenatal and postnatal lambs; if this finding were the only factor involved we would have expected to find no difference between newborn and adult inactivation. The most simple

explanation is that pulmonary bradykininase appears only late in fetal life and does not reach adult levels at birth. Whatever the explanation, it does not alter the premise that bradykinin could act as a circulating hormone in the perinatal period. This finding would be of great importance if bradykinin does participate in circulatory adaptation to extrauterine life. It has been shown that, on ventilation of fetal lambs with oxygen, large amounts of bradykinin appear in the left atrial blood with simultaneous rapid depletion in kininogen (13). The physiological effect of bradykinin would then be very short-lived after depletion of kininogen if 90% inactivation were to occur during the next passage through the lungs. Low pulmonary inactivation, however, could contribute to a longer lasting effect of bradykinin.

The lung is the most potent but not the only site of inactivation of bradykinin. For instance, its half-life in adult cat blood is only about 17 seconds (2). Two facts in our study suggest that extrapulmonary inactivation is also low in fetuses. (1) The very long half-life of the pressure response may be due to prolonged survival of bradykinin, although we recognize that a different autonomic nervous responsiveness in the fetus could also be important. (2) The pulmonary artery pressure response to aortic infusion is only slightly below that obtained after pulmonary artery infusion, indicating that very little inactivation occurs during the systemic vascular transit.

Because pulmonary bradykininase- and angiotensin I-converting enzyme are probably two functions of the same protein (18, 19) it is relevant to mention the study by Hebert et al. (15) regarding pulmonary conversion of angiotensin I in fetal and newborn lambs. Their results correlate closely with ours. The pulmonary contribution of angiotensin I conversion was less in newborn lambs than it was in adult ewes, and one premature fetus showed no apparent conversion of angiotensin I in the lungs (15). The similarity in the perinatal development of inactivation of bradykinin and activation of angiotensin I should be expected if these functions are due to a single enzyme.

Our data strongly suggest that the inactivation of bradykinin is a metabolic function of the lung which appears late in fetal life and does not reach adult level at birth. Low pulmonary extraction of bradykinin may be important physiologically if this

vasoactive peptide participates in circulatory adjustments to extrauterine life.

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