SUMMARY  Angiotensin-converting enzyme (ACE) activity in the guinea pig fetal-placental unit was assessed at the different oxygen tensions found in utero, during labor, and at birth. To determine fetal-placental ACE activity, we separately perfused in situ term guinea pig fetuses and their placentas via the umbilical vessels under controlled conditions of flow, temperature, and pH. ACE activity was defined as the percent of angiotensin I (AI) or bradykinin (BK) in Krebs-Henseleit solution cleared by a single passage through the placenta or fetus. Peptide concentrations were measured by radioimmunoassay (RIA). Using BK as substrate, we found that placental and fetal ACE activities were reflected by 45% (SD = 10) and 24% (SD = 7) clearances, respectively, at a perfusate PO2 of 29 mm Hg. Maternal hypoxia (PaO2 = 28 mm Hg) decreased placental ACE activity to 16% (SD = 8) and maternal hyperoxia (PaO2 = 191 mm Hg) increased placental ACE activity to 56% (SD = 9). Using a perfusate PO2 of 85 mm Hg, fetal and placental ACE activity increased in less than 5 minutes to 75% (SD = 10) and 77% (SD = 9), respectively. Similar results were obtained using AI as substrate. We conclude that: fetal-placental ACE activity exhibits a chronically reduced level of activity appropriate to the low oxygen tension found in the fetal-placental unit; the placenta is the primary site of ACE activity in the fetus; maternal oxygenation modulates fetal-placental ACE activity; and fetal ACE activity acutely increases with increased fetal oxygenation and thus may play an important physiological role in the regulation of circulating levels of BK and AI and the circulatory adjustments at birth. Circ Res 48: 286-291, 1981

ANGIOTENSIN II and bradykinin are potent circulating vasoactive peptides which may have important roles in fetal and neonatal blood pressure homeostasis (Mott, 1975) as well as the circulatory adjustments that occur at birth (Mellmon et al., 1968). Angiotensin-converting enzyme (kininase II) is located on the plasma membrane of vascular endothelial cells and is primarily responsible for both the activation of angiotensin I (AI) to angiotensin II (AII) as well as the degradation of bradykinin (BK). By mid-gestation, angiotensin-converting enzyme (ACE) is present throughout the fetal-placental unit of the rabbit, and histological (Wigger and Stalcup, 1978) and functional (Stalcup et al., 1978) studies indicate developmental increases to term; ACE activity in the term neonatal lung exceeds 80% of adult values under normoxic conditions. The activity of pulmonary and systemic ACE is regulated by oxygen tension. Acute hypoxia causes inhibition of enzyme activity in vivo for both AI conversion (Leuenberger et al., 1978) and BK degradation (Stalcup et al., 1979a) as well as in endothelial cell culture (Stalcup et al., 1979b). The degree of enzyme activity is closely related to PO2. Changes in enzyme activity occur rapidly with changes in PO2. It is not known how chronic low oxygen tension, as exists in the fetal-placental unit, affects ACE activity. In the studies reported here, we hypothesized that, at term, fetal-placental ACE activity is normally low due to chronic exposure to low oxygen tension but could be modulated by acute changes in oxygen tension. Thus, varying levels of ACE activity would be expected in utero, during labor, and at the time of birth. Because the placenta is the site of highest oxygen tension in the fetal-placental unit and receives 40-50% of the fetal cardiac output (Rudolph and Heymann, 1970), the placenta could make a relatively major contribution to total ACE activity in the fetal-placental unit. Therefore, we measured ACE activity separately in the placenta and fetus at normal umbilical arterial oxygen tensions, using an in situ perfusion technique. We then examined the effect of acute changes in oxygen tension on ACE activity in the fetus and placenta, thus simulating changes expected in the perinatal period.

Methods

All experiments were carried out on fetal Hartley guinea pigs and their placentas between 61 and 63 days of timed gestation (term = 65 to 68 days) to
study modulation of ACE activity by oxygen tension near term birth. Gestational age was confirmed by weighing the fetus and comparing the value on a weight-for-date table (Kaufman and Davidoff, 1977). The in situ perfusion preparation is shown in Figure 1. Anesthesia was induced with ketamine 40 mg/kg, im, and supplemented with pentobarbital 6 mg/kg, im. The maternal guinea pig was ventilated with either room air, hypoxic, or hyperoxic gas mixtures delivered by a small animal ventilator (Harvard Apparatus Co.) through a tracheostomy. A carotid arterial catheter was used to monitor maternal blood pressure, arterial blood gases, and acid-base status. The uterus was exposed by a lower midline abdominal incision. The entire preparation was then immersed into a constant 39°C saline bath to prevent umbilical vessel constriction, known to occur upon exposure to cold air (Melmon et al., 1968), to prevent air breathing by the fetus, and to keep the fetus and placenta at normal body temperature. Through a small uterotomy, a fetus was exteriorized with its umbilical circulation intact. Numbers 22 and 20 gauge angiocaths (Deseret Co.) were inserted into an umbilical artery and vein, respectively, so that the placenta could be perfused separately in the direction of normal flow. The remaining umbilical artery and two vitelline vessels were ligated.

Kreb's-Henseleit solution containing BK (Peninsula, Inc.) at 100 ng/ml or Al (Peninsula, Inc.) at 10 ng/ml was used for perfusion. These peptide concentrations were chosen because they are comparable to the levels that occur at birth (Lumbers and Reid, 1977 and Heymann et al., 1969) and are well below enzyme saturation (Stalcup et al., 1978). BK was the substrate employed in a majority of the experimental protocols with the number demonstrated in the figures. Al was employed to help confirm the specificity of the preparation for ACE (n = 4 to 8 for each experiment). The perfusate was equilibrated with either a gas mixture of low oxygen tension (3% O₂, 5% CO₂, 92% N₂) to simulate fetal umbilical arterial blood gas tensions or one of high oxygen tension (13% O₂, 5% CO₂, 82% N₂) to simulate neonatal arterial blood gas tensions. Flow was kept constant by a peristaltic infusion pump (Buchler Instruments) at 86 ml/kg per min, the estimated fetal guinea pig umbilical blood flow (Bartels et al., 1967). The perfusate was warmed to 39°C in a coil of polyvinyl tubing placed in the water bath. A three-way stopcock was placed in the perfusion line so that samples for perfusate peptide concentrations could be obtained. Inflow pressure was measured by a pressure transducer (model P23AA, Statham Co.) connected to a side arm of the umbilical catheter. After the effluent became blood free, perfusate and effluent samples for peptide concentration and maternal arterial blood samples for blood gases and pH were obtained simultaneously at each step of the protocol. Samples were frozen at ~20°C for subsequent radioimmunoassay of BK and Al by methods previously reported (Stalcup et al., 1978). The BK assay has a sensitivity of 25 pg/ml and a coefficient of variation of 10.2%. The AI assay has a sensitivity of 5 pg/ml and coefficient of variation of 13.3%.

Activity of placental or fetal converting enzyme is defined as the percent of substrate cleared by a single passage through either vascular bed. With equal perfusion inflow and effluent rates ACE activity can be calculated by the following equation:

\[
\frac{[S]_P - [S]_E}{[S]_P} \times 100 = \% \text{ substrated cleared}
\]

where [S]ₚ = substrate concentration (ng/ml) in perfusate (P) or effluent (E). Statistical comparisons were made by means of the t-test of unpaired data and regression analysis (Colton, 1974) using a Wang 600 computer. Differences in results were considered significant at a level of P < 0.05.

**Experimental Design**

**Placental Perfusion**

To assess placental ACE activity under in utero conditions, we ventilated the mother with room air and equilibrated the placental perfusate with the gas mixture containing low oxygen simulating normal umbilical PaO₂ (29 mm Hg). Simultaneous samples of perfusate, effluent, and maternal blood were taken 5 minutes after the onset of perfusion and 5 minutes after beginning each change in either maternal or perfusate O₂. To study the effect of changing maternal PaO₂ on placental ACE activity, we used the low PO₂ in the placental perfusate and altered maternal PaO₂ by ventilating the mother with either 40% O₂ or 8% O₂ (balance N₂) for 5 minutes. Measurements of placental ACE activity during maternal normoxia (control group) always

![Figure 1 Diagram of the in situ perfusion of the guinea pig fetus and placenta. UA = umbilical artery; UV = umbilical vein.](http://circres.ahajournals.org/externalgraphics/doi:10.1161/01.RES.33.3.287)
were made immediately before or after each test group (maternal hypoxia or hyperoxia). Statistical comparisons were made between a single test group and the control group. To determine the effect on ACE activity of an increase in oxygen tension on the fetal side of the placenta, the mother was ventilated with room air and the perfusate was equilibrated with the high oxygen tension gas mixture simulating neonatal Pao2 (95 mm Hg). Under the latter conditions, we also studied the specificity of the preparation for ACE activity by adding converting enzyme inhibitor SQ20,881 (Squibb & Sons, Inc.) to the perfusate containing BK to yield a final concentration of 6 ug/ml. We previously have found that this concentration of SQ20,881 will inhibit greater than 90% of ACE activity (Stalcup et al., 1979b).

Fetal Perfusion

To study the modulation of fetal ACE activity by oxygen tension, we perfused fetuses with solutions containing either BK or AI and equilibrated with either the low or high oxygen tension gas mixture. The fetal and the placental perfusion circulations were mutually exclusive, since those umbilical vessels not catheterized were ligated. Perfusate and effluent sampling as well as perfusion pressure measurements were taken 5 minutes after the onset of perfusion. This was sufficient time for the effluent to become blood free and brief enough to make measurements while fetal cardiac contractions persisted, as reflected by the pulsatile effluent flow and upon direct observation during the subsequent dissection. The perfusion was stopped after 5 minutes and each fetus was weighed to assess gestational age and dissected immediately to confirm complete displacement of blood in the major organs. The specificity of this preparation also was assessed: three fetuses were studied using the perfusate containing BK and high oxygen tension with SQ20,881 at 6 µg/ml.

Results

Perfusion Preparation

The mean fetal weight of the total population studied was 90 ± 10 (mean ± SD) g, indicating that the mean gestational age was 63 days (term 65–68). Perfusate Pao2 values resulting from the equilibration of the perfusate with low or high oxygen tension gas mixtures were 29 ± 3 mm Hg or 95 ± 4 mm Hg, respectively; the pH (7.34 ± 0.03) and Pco2 (43 ± 5 mm Hg) equilibrating with the low oxygen mixture were not significantly different from the pH (7.35 ± 0.07) and Pco2 (43 ± mm Hg) equilibrating with the high oxygen mixture, (P > 0.7). Mean perfusate and effluent flow rates were identical (86 ± 4 ml/kg per min). During placental perfusions, maternal mean blood pressure was 59 ± 14 mm Hg with the mother breathing room air. Although phasic increases in maternal blood pressure were observed with the onset of maternal hypoxia, there were no significant differences in maternal blood pressure 5 minutes after a change in maternal oxygenation. Mean placental perfusion pressure in the umbilical artery was 45 ± 14 mm Hg and was not affected by the perfusate oxygen tension or type of peptide substrate employed; mean fetal perfusion pressure in the umbilical vein was 24 ± 4 mm Hg and also was not significantly affected by either perfusate oxygen tension or peptide substrate (P > 0.2).

Effect of Changes in Fetal and Maternal Pao2 on ACE

When the perfusate Pao2 simulated that normally found in the fetal umbilical artery (Pao2 = 29 mm Hg), placental ACE activity was 45 ± 10% with BK as the substrate (Fig. 2) and 42 ± 7% when AI was used. Fetal ACE activity was significantly less (P < 0.001) being 24 ± 7% with BK as substrate (Fig. 2) and 16 ± 14% when AI was used. Figure 3 illustrates the changes in placental ACE activity observed with changes in maternal oxygenation using BK as substrate. Maternal hypoxia (Pao2 = 28 ± 5 mm Hg) resulted in a significant decrease (P < 0.001) in ACE activity from 45% to 16 ± 8% and 42% to 10 ± 8% with BK and AI, respectively as substrates. With moderate maternal hyperoxia (Pao2 = 192 ± 48 mm Hg), placental ACE activity significantly increased (P < 0.02) from 43% to 56 ± 9% (BK substrate). The highest level of fetal-placental ACE activity occurred when the high oxygen tension perfusate (Pao2 = 95 mm Hg) was used to simulate a normal neonatal Pao2: fetal ACE activity was significantly greater than 90%. Figure 3 illustrates the changes in placental ACE activity observed with changes in maternal oxygenation using BK as substrate. Maternal hypoxia (Pao2 = 28 ± 5 mm Hg) resulted in a significant decrease (P < 0.001) in ACE activity from 45% to 16 ± 8% and 42% to 10 ± 8% with BK and AI, respectively as substrates. With moderate maternal hyperoxia (Pao2 = 192 ± 48 mm Hg), placental ACE activity significantly increased (P < 0.02) from 43% to 56 ± 9% (BK substrate). The highest level of fetal-placental ACE activity occurred when the high oxygen tension perfusate (Pao2 = 95 mm Hg) was used to simulate a normal neonatal Pao2: fetal ACE activity was significantly greater than 90%.
CONVERTING ENZYME ACTIVITY IN THE GUINEA PIG FETUS/Davidson et al.

70 -
60 -
50 -
40 -
30 -
20 -
10 -

FETAL Pao2 (mmHg) 29
MATERNAL Pao2 (mmHg) 28

MATERNAL HYPOXIA
MATERNAL NORMOXIA
MATERNAL HYPEROXIA

activity significantly increased (P < 0.001) from 24% to 75 ± 10% with BK as substrate (Fig. 4) and 16% to 58 ± 10% using AI. Employing BK as substrate, placental ACE activity increased from 45% to 77 ± 9% (Fig. 4).

We confirmed the specificity of the perfusion methods for ACE activity by adding SQ20,881 to the high oxygen tension perfusate containing BK:

FIGURE 3 The effect of changing maternal Pao2 on ACE activity in the guinea pig placenta. Statistical comparisons were made between values from one test group and control values obtained when the mother was breathing room air (Pao2 = 83 mm Hg).

activity significantly increased (P < 0.001) from 24% to 75 ± 10% with BK as substrate (Fig. 4) and 16% to 58 ± 10% using AI. Employing BK as substrate, placental ACE activity increased from 45% to 77 ± 9% (Fig. 4).

We confirmed the specificity of the perfusion methods for ACE activity by adding SQ20,881 to the high oxygen tension perfusate containing BK:

FIGURE 4 Demonstration of the increase in ACE activity which occurs in both the guinea pig fetus and placenta within 5 minutes of achieving neonatal Pao2 (95 mm Hg). Statistical comparisons were made between values of ACE activity at low and high oxygen tension separately for the fetus and placenta.

placental and fetal ACE activity dropped to 11 ± 5%. When we used either substrate or perfusate oxygen tension, we found no correlation between perfusion pressure and ACE activity. Thus the levels and changes in fetal-placental ACE activity observed were primarily due to modulation by Po2 and not to a change in vascular surface area.

Discussion

These experiments indicate that converting enzyme activity, in both the fetus and placenta, is modulated by changes in either fetal or maternal Pao2 likely to occur in the perinatal period. The fetal-placental unit is normally exposed to a low oxygen tension and, as a result, fetal-placental ACE activity is reduced when compared to pulmonary and systemic ACE activity in the neonatal and adult animals studied at a Po2 greater than 95 mm Hg (Stalcup et al., 1978, 1979a). However, in spite of the chronic inhibition of ACE activity in utero, fetal-placental ACE activity can be modulated rapidly by acute changes in oxygen tension. During labor there may be appreciable alterations in maternal Pao2 or utero-placental blood flow resulting in a change in oxygen transfer across the placenta and thus a change in fetal-placental ACE activity. In addition, the birth process results in an increase in oxygenation and thus a rapid increase in systemic and pulmonary ACE activity of the newborn.

The in situ placental perfusion system is a conventional model for the study of placental metabolism (Money and Dancis, 1960) which we adapted to study angiotensin-converting enzyme (kininase II) in the fetus and placenta. The guinea pig has a discoid hemomonochorial placenta structurally similar to that of humans (Kaufman and Davidoff, 1977). The use of a blood-free perfusion system eliminates the relatively slow and oxygen-insensitive reactions of circulating kininase (kininase I) from these experiments (Stalcup et al., 1979a). Since AI and presumably AI and BK do not cross the placenta (Symonds, 1979), the clearance of the latter two peptides from the umbilical artery to vein would be expected to be dependent on placental ACE activity. This hypothesis was supported by the low clearance of substrate after inhibition of placental ACE activity by SQ20,881. The fetal preparation does not permit us to assess the importance of shunting on our measurements of fetal ACE activity. There are several reasons which lead us to believe that our values of fetal ACE activity reasonably approximate the level in the intact fetus. The fetus in our preparation is perfused by the umbilical vein, and gross autopsy always revealed complete replacement of blood in all major organs including the skin and lungs where vasoconstriction would be likely to occur. The two substrates of ACE, angiotension and bradykinin, often have opposite effects on the local distribution of blood flow; yet when we used physiological concentrations of these substrates, we obtained similar values and the same...
direction of change for ACE activity at various levels of oxygen tension.

The placenta and fetus were perfused separately so that we might assess the relative contribution placental ACE activity makes to the total enzyme activity in the fetal-placental unit. Using a perfusate PO₂ which approximates fetal PO₂ under normal in utero conditions, we found that ACE activity in the placenta was higher than in the fetus presumably because of the higher PO₂ in the placenta. This was supported by the observation that, when the maternal Pao₂ was reduced to the range normally present in the fetus, placental ACE activity decreased to approximately the same level as fetal ACE activity. We conclude that, since the placenta receives 40-50% of the fetal cardiac output and has a relatively high ACE activity, the placenta is the principal site of ACE activity in the fetal-placental unit. In addition, placental ACE activity is sensitive to changes in maternal oxygenation. The fetal pulmonary circulation receives only 5-10% of the fetal cardiac output and receives most of its blood flow from the poorly oxygenated superior vena caval return (Rudolph and Heymann, 1970). Therefore, the fetal pulmonary circulation would not be expected to be a major site of ACE activity, in contrast to its central role in the handling of these and other vasoactive substances after birth (Fishman and Pietra, 1974).

Our work demonstrating that the placenta is the principal site of fetal-placental ACE activity taken in conjunction with the work of others (Mott, 1975; Iwamoto and Rudolph, 1979), who have shown the importance of the renin-angiotensin system in fetal blood pressure homeostasis suggests that the placenta may play an important role in fetal blood pressure regulation, umbilical blood flow, and the distribution of fetal-placental blood volume. Based on the developmental increases in ACE and its activity during gestation (Wigger and Stalcup, 1978; Stalcup et al., 1978), one would expect placental ACE activity to play an increasingly important role in the production of fetal All. This developmental process correlates well with the rise in fetal blood pressure during gestation (Rudolph and Heymann, 1970) and, in turn, would ensure an increase in umbilical blood flow to the low resistance placental vascular bed in proportion to fetal growth.

Appreciable changes in maternal Pao₂ may occur during labor, especially in response to analgesics or sedatives administered to the mother. In addition, alterations in utero-placental blood flow associated with pathological decelerations of fetal cardiac rate will result in a decrease in oxygen delivery to the fetus (Huch et al., 1977). These pathological events will lead to fluctuations in fetal-placental PO₂ and consequently ACE activity, resulting in parallel changes in circulating All and BK levels in the fetus. We expect that hypoxic inhibition of ACE activity will predispose the fetus to the risk of hypotension and result in pathological redistribution of blood flow. Furthermore, activation of the kallikrein-kinin system by acidosis (O'Brodovich et al., 1980) in the fetal-placental unit might exacerbate these hemodynamic changes due to an increase in fetal arterial (BK). To some degree, maternal hyperoxia may increase fetal-placental ACE activity, and this would be likely to increase circulating levels of All and decrease circulating levels of BK. However, maternal hyperoxia will result in a limited increase in fetal-placental PO₂ because of the high placental oxygen consumption (Battaglia et al., 1968). The extent to which circulating levels of All and BK can be altered by an increase in maternal oxygenation remains to be elucidated.

The increase in ACE activity resulting from the increase in oxygenation at birth could play an important role in the regulation of circulating levels of BK and All, and thus in the circulatory adjustments at birth. Bradykinin is liberated from the lung on exposure of fetal leukocytes to an increase in oxygen tension at birth (Melmon et al., 1968; Heymann et al., 1969). Since bradykinin is a pulmonary vasodilator and constrictor of the ductus arteriosus and umbilical vessels, it has been implicated as a mediator of perinatal circulatory adjustments. The present study indicates that, after liberation, BK will be degraded in proportion to the rise in oxygen tension and ACE activity at birth. Although the renin-angiotensin system plays an important role in fetal and neonatal blood pressure homeostasis (Mott, 1975), there have been no studies which describe circulating levels of All and its function in the minutes immediately after birth. It is known, however, that the cord level of plasma renin activity is elevated (Lumbers and Reid, 1977). Thus the large increase in ACE activity seen with increase in oxygenation of the fetus in the present study suggests that All will be rapidly generated at birth.

We believe that the oxygen-dependent increase in All generation and BK degradation may play an important role in the development of the high systemic vascular resistance required for the normal circulatory adjustments at birth (Assali et al., 1965), when arterial levels of BK are transiently high. However, lack of adequate oxygenation at birth in combination with a developmental impairment of pulmonary ACE activity (Stalcup et al., 1978) may lead to a decrease in All generation and prolongation of high arterial levels of BK in the hypoxic premature neonate. The effect of low All and high BK arterial levels immediately after birth would be expected to contribute to known pathophysiological changes of the circulation associated with neonatal respiratory distress syndrome, namely, hypotension, right-to-left shunting, hypoproteinemia, and peripheral and pulmonary edema (Stalcup and Melins, 1980).

The contributions of BK and All, as "first messenger" circulating vasoactive substances, to normal or pathophysiological adjustments of the pul-
monary and systemic circulation at birth may be mediated directly or via the interaction with “second messengers” such as cyclic nucleotides (Goldberg, 1975) and prostaglandins (Needleman et al., 1975). Angiotensin II and bradykinin are known to be potent inducers of phospholipase A₂ and hence important in the production of arachidonic acid. Therefore, in the lung, these peptides may stimulate the production of PGI₂ (Moncada et al., 1978), which is known to have a potent vasodilatory effect on the pulmonary circulation in the neonatal period (Lock et al., 1979). Thus the modulation of ACE activity by oxygen tension at the time of birth may serve as a finely graded mechanism for regulating arterial concentrations of “first” and possibly “second messenger” vasoactive mediators involved in the circulatory adjustments at birth.

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