Altered fetal cardiovascular responses to prolonged hypoxia after sinoaortic denervation

P. Stein, S. E. White, J. Homan, M. A. Hanson, and A. D. Bocking. Altered fetal cardiovascular responses to prolonged hypoxia after sinoaortic denervation. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R340–R346, 1999.—This study examines the role of the peripheral chemoreceptors in mediating fetal cardiovascular responses to prolonged hypoxia secondary to reduced uterine blood flow (RUBF). Fetal sheep were chronically instrumented for continuous heart rate (FHR), blood pressure (FBP), and carotid blood flow (CBF) measurements after bilateral sectioning of the carotid sinus and vagus nerves (denervated, n = 7) or sham denervation (intact, n = 7). Four days postoperatively, uterine blood flow was mechanically restricted, reducing fetal arterial oxygen saturation by 47.3% (P < 0.01). An initial bradycardia was observed in intact (184.0 ± 10.7 beats/min, not significant) but not denervated fetuses, followed by a tachycardia (180.0 ± 2.2 to 193.7 ± 27 beats/min, P < 0.05). FHR increased in denervated fetuses (175.5 ± 8.7 to 203.0 ± 17.9 beats/min, P < 0.05). FBP increased transiently in intact fetuses from 45.1 ± 1.0 to 55.4 ± 3.0 mmHg at 2 h (P < 0.01), whereas denervated fetuses demonstrated a decrease in FBP from 47.1 ± 4.2 to 37.2 ± 3.7 mmHg (not significant). CBF increased (P < 0.05) in both intact and denervated fetuses from 39.3 ± 2.8 and 29.7 ± 3.8 ml·min⁻¹·kg⁻¹ to 47.7 ± 0.4 and 39.1 ± 0.3 ml·min⁻¹·kg⁻¹, respectively, whereas carotid vascular resistance decreased only in denervated fetuses (1.7 ± 0.1 to 1.1 ± 0.02 mmHg·ml⁻¹·min⁻¹·kg⁻¹, P < 0.05). We conclude that the peripheral chemoreceptors play an important role in mediating fetal cardiovascular responses to prolonged RUBF.

Peripheral chemoreceptors act as sensory receptors relaying information to the brain stem related to changes in arterial Po₂, PCO₂, and pH. Their possible role in mediating fetal cardiovascular responses to acute hypoxia has been extensively studied. Itskovitz and Rudolph (18) reported that sinoaortic denervation abolishes the initial bradycardia and transient increase in arterial blood pressure during acute hypoxia and that the peripheral vasoconstriction and CVO redistribution are attenuated (16, 19). Giussani et al. (9) investigated the role of the carotid chemoreceptors exclusively and showed that the initial bradycardia and increase in femoral vascular resistance are primarily carotid chemoreflexes.

The physiological processes controlling the cardiovascular responses to more prolonged episodes of fetal hypoxia are currently unknown. These studies were therefore designed to examine the role of the peripheral chemoreceptors in mediating fetal cardiovascular responses to prolonged hypoxia, secondary to 24 h of reduced uterine blood flow (RUBF). In addition, we wished to determine the role of the peripheral chemoreceptors in mediating the endocrine and growth changes within the fetus in response to prolonged RUBF (4, 12, 13). It was therefore critical in this series of experiments to ensure that both the carotid and aortic chemoreceptors were denervated. Numerous methods have been used previously to selectively denervate the peripheral chemoreceptors. Carotid body denervation involves sectioning the carotid sinus nerve and/or stripping nervous and connective tissue rostral and caudal to the origin of the lingual and occipital arteries (9, 18). Selective denervation of the aortic body, however, is more complex. Both midcervical vagotomy (19) and sectioning of the aortic and superior laryngeal nerves (18) are standard methods that have been used to denervate the aortic body, although neither is ideal. Midcervical vagotomy also interrupts cardiopulmonary and great vessel afferents in addition to cardioinhibitory efferents, whereas the aortic nerve itself may also contain pulmonary mechanoreceptors (8). Furthermore, the aortic branch is not always separate from the vagus nerve in the newborn lamb, and therefore some aortic chemoreceptor afferents run in the main vagus (21). Because the current study is the first to examine the role of the peripheral chemoreceptors in mediating fetal responses to prolonged hypoxia, midcervical vagotomy was performed in addition to carotid sinus denervation to ensure complete peripheral chemoreceptor denervation.

Fetal cardiovascular responses to episodes of reduced oxygenation have been extensively studied using the chronically catheterized ovine fetus. In fetal sheep of >110 days of gestation, cardiovascular responses to acute hypoxia include an initial bradycardia (3, 6), increased heart rate variability (22), increased arterial blood pressure (3, 6), increased femoral vascular resistance (6), and a redistribution of combined ventricular output (CVO) favoring the cerebral, myocardial, and adrenal vascular beds (6, 23). During more prolonged periods of hypoxia, the initial bradycardia is followed by a sustained tachycardia that is maintained for 12–16 h (1). Fetal heart rate (FHR) accelerations and decelerations increase initially and return to normal patterns indistinguishable from normoxic fetuses (2). Arterial blood pressure increases transiently followed by a return to normoxic values, whereas CVO redistribution is maintained for at least 48 h (1).
MATERIALS AND METHODS

Surgical procedures. Surgery was performed on 14 pregnant sheep of known mating dates between 118 and 126 days of gestation, with a mean (±SE) gestational age at the time of surgery of 121.9 ± 0.7 days. Anesthesia was induced using intravenous thiopental sodium (Abbott Laboratories, Montreal, Quebec) and maintained with 1.0–1.5% halothane (Halocarbon Laboratories, Hackensack, New Jersey) in oxygen at a flow rate of 5–6 l/min. Under sterile conditions, a midline abdominal incision was made and a polytetrafluoroethylene vascular clamp was placed around the maternal common internal iliac artery. Using the technique described by Giussani et al. (9), we performed bilateral carotid denervation followed by midcervical vagotomy bilaterally in seven fetuses, which are termed “intact” fetuses. Polyvinyl catheters (V4; Bolab, Lake Havasu City, Arizona) were placed in the fetal carotid artery for the experimental period. Those nerves were identified and left uncut in seven fetuses, which are termed “denervated” fetuses. These nerves were exteriorized through the maternal flank.

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Experimental protocol. All experiments began between 9:00 and 10:00 AM, with a 2-h control period during which FHR, arterial blood pressure, and CBF were measured continuously. At time 0, the vascular clamp was released and the maternal carotid artery was exteriorized through the maternal flank. Sodium penicillin G (1 × 10^6 U) was infused into the fetus at the time of surgery and then daily for 3 days. Sodium penicillin G (8 × 10^5 U) and streptomycin (100 mg) (Pen-di-Strep; Rogar/STB, London, Ontario) were injected intramuscularly into the ewe at the same time intervals. Animals were housed in individual cages with free access to food and water and allowed a minimum of 4 days to recover from surgery before experiments commenced. All animals were treated in compliance with guidelines established by the Canadian Council on Animal Care and according to protocols approved by the Animal Care Committee of the Lawson Research Institute and the University of Western Ontario.

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RESULTS

Blood gases and arterial SaO2. During the 2-h normoxia period, blood gases and SaO2 were similar in both groups. After the onset of RUBF, SaO2 decreased significantly (P < 0.01) in both intact and denervated fetuses from 62.4 ± 5.0 and 60.3 ± 6.1% to 35.4 ± 2.7 and 28.2 ± 2.0%, respectively (Fig. 1A). A similar decrease in arterial Po2 was observed in both groups, whereas arterial Pco2 remained unchanged (Fig. 1, B and C). Arterial pH decreased significantly at 1 h in both groups as a result of a transient decrease in base excess followed by a return toward control values at 12 h (Fig. 2, A and B). At 20 and 24 h, arterial pH was significantly lower (P < 0.05) in denervated fetuses compared with the control period; however, it was not significantly different from intact fetuses (Fig. 2A). Plasma lactate concentrations remained significantly elevated (P < 0.01) throughout the 24-h RUBF period in intact fetuses, and denervated fetuses demonstrated a progressive increase in lactate concentrations, reaching statistical significance at 24 h of RUBF only (Fig. 2C). There was no change in either glucose or hemoglobin concentrations during these experiments in either group of animals.

FHR and arterial blood pressure. With the onset of RUBF, FHR decreased in intact animals from 184.0 ± 10.7 beats/min 5 min before the restriction in uterine blood flow to 160.5 ± 10.7 beats/min at the onset of RUBF, although this was not statistically significant. FHR then significantly increased (P < 0.05) from a mean value of 180.0 ± 2.2 beats/min during the 2-h control period to a mean value of 193.7 ± 2.7 beats/min during the initial 16 h of RUBF, followed by a return to
control values (Figs. 3A and 4A). Compared with intact fetuses, FHR initially increased \( (P < 0.05) \) in denervated fetuses from 175.5 ± 8.7 beats/min 5 min before the restriction in uterine blood flow to a maximum value of 203.0 ± 17.9 beats/min at 5 min (Fig. 3A). Overall, FHR increased in denervated fetuses from 165.2 ± 5.6 beats/min during the 2-h control period to 176.8 ± 0.9 beats/min throughout the 24-h RUBF period, although this was not statistically significant (Fig. 4A).

Arterial blood pressure increased in intact fetuses from 44.2 ± 1.1 mmHg 5 min before RUBF to 50.8 ± 1.0 mmHg 5 min after the onset of RUBF, although this was not statistically significant. Arterial blood pressure increased \( (P < 0.01) \) transiently in intact fetuses to a maximum value of 55.4 ± 3.0 mmHg at 2 h, followed by a return to control values. There was no consistent change in arterial blood pressure in denervated fetuses with the onset of RUBF, as a result of considerable variability both within and between animals (Fig. 3B). Arterial blood pressure decreased from 47.1 ± 4.2 to 37.2 ± 3.7 mmHg at 2 h of RUBF and remained low throughout the 24-h RUBF period, although this was not statistically significant (Fig. 4B). Arterial pressure variability, as measured by the coefficient of variation, was significantly greater \( (P < 0.001) \) throughout the 2-h control and 24-h RUBF periods in denervated fetuses (12.6 ± 0.6%) compared with intact fetuses (6.2 ± 0.8%).

Carotid vascular resistance and blood flow. Under normoxic conditions, carotid vascular resistance was greater \( (P < 0.05) \) in denervated fetuses (1.7 ± 0.1 mmHg·ml⁻¹·min⁻¹·kg⁻¹) compared with intact fetuses (1.2 ± 0.01 mmHg·ml⁻¹·min⁻¹·kg⁻¹). With the onset of RUBF, carotid vascular resistance decreased \( (P < 0.05) \) in denervated fetuses to 1.1 ± 0.0 mmHg·ml⁻¹·min⁻¹·kg⁻¹, whereas in intact fetuses there was no change (Fig. 5A). Overall, CBF was significantly lower \( (P < 0.05) \) in denervated fetuses compared with intact fetuses throughout the 2-h control and 24-h RUBF periods. CBF increased \( (P < 0.05) \) in both intact and denervated fetuses with the onset of RUBF from 39.3 ± 2.8 and 29.7 ± 3.8 ml·min⁻¹·kg⁻¹ to 47.7 ± 0.4 and 39.1 ± 0.3 ml·min⁻¹·kg⁻¹, respectively, representing an increase of 18 and 24% (Fig. 5B).

**DISCUSSION**

This study is unique in that it examines for the first time the role of the peripheral chemoreceptors in mediating fetal cardiovascular responses to prolonged hypoxia. FHR was lower throughout the 24-h RUBF
period in denervated fetuses compared with intact fetuses. Arterial blood pressure decreased with RUBF in denervated fetuses in association with a decrease in carotid vascular resistance. Carotid vascular resistance was greater and CBF was lower in denervated fetuses under control conditions, although CBF increased to the same extent during RUBF in both groups of animals.

In investigating the neural components of these responses, we used the technique of denervation. In addition to determining the combined effects of chemodenervation and RUBF on cardiovascular function, we also wished to examine the role of the peripheral chemoreceptors in mediating the endocrine and growth fetal adaptive responses to prolonged RUBF. These results have been reported separately (27). Therefore, to ensure complete peripheral chemoreceptor denervation, we sectioned the carotid sinus and vagus nerves bilaterally. Although our primary objective was to examine the role of afferent input to the brain, we recognize that the technique of cutting the vago-sympathetic trunk also removes the influence of cardiopulmonary and great vessel afferents as well as vagal and sympathetic efferents.

Before performing these experiments, we considered sectioning the aortic nerves selectively; however, the aortic nerve is not always separate from the vagus nerve in the newborn lamb, and some aortic body afferents run in the main vagus (21). Adequate selective denervation of the aortic bodies necessitates stripping branches of the aortic nerve from the great arteries in the chest, and we felt that the extent of this surgery would be too great to be justified. The loss of the rapid bradycardia at the onset of hypoxia in our denervated animals could therefore be due to either the absence of carotid chemoreceptor input to the brain stem or the loss of vagal cholinergic innervation of the heart (9). In addition, the differences in CBF and carotid vascular resistance observed between denervated and intact fetuses in these experiments may be due to the removal of sympathetic efferent fibers coursing to the head in denervated animals. Wagerle et al. (29) reported that the fetal cerebral circulation is highly sensitive to norepinephrine, suggesting a regulatory role for sympathetic nerves in the immature fetal period.
be due either to the absence of carotid body afferents or the loss of vagal cholinergic innervation of the heart. After carotid sinus denervation alone, however, arterial blood pressure does not increase after the onset of acute hypoxia (9). In our study, arterial blood pressure also did not increase in denervated fetuses after the onset of RUBF, indicative of complete carotid sinus denervation. We are confident that sectioning of the vagus nerve was complete because a 2- to 3-mm portion of the nerve was removed and the ends were identified. Experiments were performed 4 days postoperatively, making it unlikely that neural regeneration would have occurred.

A similar decrease in fetal arterial Po₂ and SaO₂ was observed in intact and denervated fetuses with the onset of RUBF, and this was maintained throughout the 24-h period. In association with this fall in SaO₂, there was a transient decrease in arterial pH that had been observed previously in our laboratory (1). Fetal arterial pH returned to normoxic values by 12 h in intact fetuses, whereas in denervated fetuses, pH remained significantly lower at 20 and 24 h compared with the normoxia period. This decrease in arterial pH was associated with a progressive increase in plasma lactate concentrations to a maximum value of 9.8 mmol/l at 24 h in denervated fetuses compared with 4.8 mmol/l in intact fetuses. Previous studies have observed that plasma lactate concentrations increase to maximal levels at −4 h of RUBF (12, 14, 30). The stabilization in plasma lactate concentrations has been attributed to an increase in lactate clearance from the fetal circulation via the kidney and placenta as opposed to an increase in lactate metabolism (5, 15). In denervated fetuses, lactate levels rose progressively throughout the 24-h RUBF period, suggesting a possible impairment in lactate clearance. Jansen et al. (19) have investigated the control of organ blood flow during normoxia and acute hypoxia in fetal sheep after vagotomy and sinoaortic denervation. Although with either vagotomy or sinoaortic denervation the distribution of CVO under normoxic conditions is not altered, both procedures affect the fetal circulatory responses to acute hypoxia. Placental blood flow is significantly reduced during acute hypoxia in both vagotomized and sinoaortic-denervated fetuses, whereas renal blood flow does not change. It is thus possible that in the present study, placental blood flow was reduced in denervated fetuses throughout the RUBF period, leading to a decrease in lactate clearance.

We have shown in this study that bilateral sectioning of the carotid sinus and vagus nerves markedly alters fetal cardiovascular responses to prolonged hypoxia secondary to RUBF. These observations extend those of previous investigators who demonstrated a role for the peripheral chemoreceptors in mediating fetal cardiovascular responses to acute hypoxia (9, 16, 18, 19). The sustained tachycardia observed previously in intact fetuses with RUBF is thought to be secondary to β-adrenergic stimulation associated with a sustained rise in plasma catecholamine concentrations (1). The attenuated tachycardia observed in denervated fetuses...
would thus suggest a decrease in β-adrenergic stimulation of the heart. In addition, the transient rise in arterial blood pressure observed in intact fetuses is in keeping with a predominance of vasoconstriction within certain vascular beds in the fetus. In contrast, the sustained hypotension observed in denervated fetuses is in keeping with a net vasodilatation, reflecting possible changes in fetal systemic vasoconstrictor mechanisms including α-adrenergic activity and/or arginine vasopressin (AVP). It is therefore likely that, under conditions of prolonged hypoxia, the fetus relies primarily on changes in vasoactive hormones in maintaining the adaptive cardiovascular responses, which are in turn regulated by peripheral chemoreceptor function. This hypothesis is supported by the findings of an attenuation of the increase in plasma AVP and catecholamine concentrations in sinoaortic-denervated fetuses with prolonged RUBF (27).

It is likely that with our technique of denervation, we also removed afferent fiber input from sensory receptors other than the carotid and aortic chemoreceptors. Although there is no evidence for a role of vagal afferents from cardiac and pulmonary receptors in mediating fetal cardiovascular responses to hypoxia, a contribution from the aortic and carotid baroreceptors cannot be excluded. Carotid vascular resistance was greater and CBF was lower in denervated fetuses under normoxic conditions. These differences may reflect a decrease in sensory afferent input after arterial baroreceptor denervation with a resulting vasodilatation. A continuous assessment of changes in combined cerebral and extracerebral blood flow was established in the present study by means of transit-time ultrasound-derived changes in carotid artery blood flow. Previous studies have demonstrated that this technique provides a reliable, continuous assessment of both cerebral and extracerebral blood flow in fetal sheep (7, 10, 28). A limitation of the use of transit-time flow transducers on the carotid artery in fetal sheep, however, is the inability to identify changes to blood flow in the cerebral and extracerebral vascular beds independently of each other. Previous studies examining regional distribution of blood flow in fetal sheep under conditions of reduced oxygenation have reported a significant increase in cerebral blood flow, with redistribution favoring the brain stem and subcortex (1, 19, 20, 25, 26). In contrast, blood flow to the peripheral tissues decreases during acute hypoxia in association with an increase in peripheral vascular resistance (20, 25, 26). Jansen et al. (19) have demonstrated an attenuation of the increase in blood flow to the brain, heart, and adrenal gland during acute hypoxia after vagotomy and sinoaortic denervation. Although attenuated, blood flow to the brain stem continued to increase in sinoaortic-denervated fetuses at the expense of the cerebrum and cerebellum. Furthermore, blood flow to the skeletal muscles increased in sinoaortic-denervated but not vagotomized fetuses, suggesting a carotid chemoreceptor-mediated change in peripheral blood flow and vascular resistance. Giussani et al. (9) reported that the femoral vasoconstriction associated with acute hypoxia is abolished after carotid sinus denervation. In contrast, there is no effect of carotid sinus denervation on the increase in CBF with acute hypoxia, suggesting that this is not carotid chemoreceptor mediated. Our study extends these observations by confirming that the peripheral chemoreceptors do not mediate the increase in CBF under conditions of prolonged hypoxia secondary to RUBF.

We conclude that the carotid sinus and vagus nerves are important in mediating fetal cardiovascular responses to prolonged RUBF in the late-gestation ovine fetus. These responses may be mediated indirectly via the peripheral chemoreceptors through changes in circulating vasoactive hormones. Further studies are warranted to determine the relative contributions of the carotid and aortic bodies in mediating these responses.

These findings have important implications for understanding the mechanisms whereby the fetus adapts to prolonged hypoxia. These adaptive mechanisms may in turn influence the ability of the fetus and, subsequently, neonate to mount the appropriate protective responses to further reductions in oxygen delivery, hypotension, or hemorrhage.

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