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Cerebral Responses to Maternal Cocaine Injection in Immature Fetal Sheep

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ABSTRACT

Previous studies in near-term sheep have shown that maternal cocaine injection causes acute fetal cerebral vasodilation along with transient hypoxemia and hypertension. Preterm sheep fetuses have lower cerebral O_2 consumption ($CMRo_2$) and their cerebrovascular responses to hypoxemia are attenuated compared with near-term fetuses. We therefore tested the hypothesis that fetal cerebrovascular responses to maternal cocaine injection may also differ earlier in gestation. We studied nine immature fetal sheep at 0.65 gestation using the same experimental protocol we used in previous studies in near-term sheep. Fetal studies were done *in utero*, 2 d after vascular catheter placement. We measured cerebral blood flow (CBF) using microspheres, arterial and sagittal sinus O_2 content, and cocaine concentrations. We calculated cerebrovascular resistance (CVR) as mean arterial blood pressure ÷ CBF. Measurements were made before and 2, 5, and 15 min after a 2 mg/kg maternal cocaine injection. At 2 min, fetal Cao_2 decreased (18 ± 6%, mean ± SEM), and there was cerebral vasoconstriction (CVR increased by 22 ± 5%). At 5 min, CBF increased (19 ± 9%), but because blood pressure increased also, CVR returned to baseline, and therefore there was no vasodilation compared with baseline. Furthermore, at 5 min there was a 22 ± 6% decrease in Cao_2 and a 21 ± 6% increase in mean arterial blood pressure. There were no changes in $CMRo_2$ throughout the study, but at 2 min, cerebral O_2 delivery decreased. Differences in cerebrovascular responses to maternal cocaine injection earlier in gestation may be due to differences in vascular development and/or to developmental differences in responses to cocaine, cocaine metabolites, and/or to hypoxemia. (***Pediatr Res* 38: 943–948, 1995**)

Abbreviations

CBF, cerebral blood flow
$CMRo_2$, cerebral oxygen consumption
CVR, cerebrovascular resistance
Cao_2, arterial oxygen content
MAP, mean arterial blood pressure

Cocaine abuse by pregnant women continues to be a major sociologic and medical problem in the United States (1, 2). Because cocaine can cause serious neurologic problems in adults, concern has been raised regarding the effects of maternal cocaine abuse on the developing brain. Although a characteristic pattern of neonatal neurologic injury and abnormal neurodevelopmental outcome has not yet emerged, clinical studies have reported an association of maternal cocaine abuse with fetal and neonatal neurologic injuries, including cerebral hemorrhages and infarctions, neurobehavioral abnormalities, and small head size (3–6). However, the pathogenesis of these defects as well as cocaine's more subtle effects on neurodevelopment is poorly understood.

Neurophysiologic studies in developing sheep, pigs, and cats have yielded conflicting results regarding the cerebrovascular effects of cocaine (7–13). We previously reported cerebral vasodilation (in some brain regions) along with hypertension and hypoxemia in near-term fetal sheep, 5 min after a 2 mg/kg maternal cocaine injection (12), and we have also reported cerebral vasodilation despite less hypoxemia after a direct fetal cocaine injection near term (14). However, there is little or no information regarding the cerebrovascular effects of cocaine on the more immature brain.

Previous studies have demonstrated that immature fetal sheep have lower CBF and $CMRo_2$ and blunted responses to hypoxemia compared with near-term fetal sheep (15, 16, 40). We hypothesized that in preterm fetuses, cerebrovascular responses to maternal cocaine could be attenuated either because of incomplete vascular development or because of blunted responses to hypoxemia. The preterm fetal brain is of considerable clinical interest because maternal cocaine abuse is associated with a higher risk of preterm labor and low birth weight (17) as well as uteroplacental abnormalities including both chronic and acute abruptio placenta with consequent fetal or perinatal asphyxia (18–20). Discovery of maturity-

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dependent fetal vascular effects of maternal cocaine abuse could account, in part, for the conflicting results in different species.

METHODS

Subjects. Nine mixed-breed fetal sheep were obtained from time-dated pregnancies verified by abdominal ultrasound. The fetuses ranged from 92 to 94 d of gestation (term = 145–150 d) at the time of study (mean 93 ± 1 d) and weighed 832 ± 29 g. Three of the fetuses were one of twins and four were females. All surgical and experimental procedures were approved by our institutional Animal Care and Use Committee.

Surgical preparation. One day before surgery, food was withheld from the ewe, although she was allowed free access to water. On the day of surgery, the ewe was anesthetized with halothane (1–2%) and an i.v. infusion of 5% dextrose in 0.45% NaCl was begun via a catheter placed percutaneously in the jugular vein. A catheter was placed in the maternal abdominal aorta via the femoral artery. The uterus was exposed through a midline abdominal incision. Small uterine incisions were used to gain access to the fetus, and catheters were placed into the superior sagittal sinus, brachiocephalic trunk (via axillary arteries), and inferior vena cava (via pedal veins) by previously described methods (15). All vascular catheters were flushed and filled with heparin (100 U/mL). Uterine and abdominal incisions were closed, and all catheters were exteriorized through the ewe's flank. Ampicillin (500 mg) was instilled into the amniotic cavity through a catheter (Tygon tubing) placed there at the end of surgery. Benzathine and procaine penicillin (1 200 000 U) were administered intramuscularly to the ewe just before surgery. The ewe was fed a standard diet of hay and water and allowed a 48-h recovery period before physiologic studies of the fetus *in utero*.

Physiological measurements. Blood flow was measured with the radiolabeled microsphere technique and the least-squares method of differential spectroscopy (21). Approximately 800 000 microspheres (0.3 mL) labeled with ^{153}Gd, ^{114}In, ^{113}Sn, ^{103}Ru, ^{95}Nb, or ^{46}Sc (DuPont NEN, Boston, MA) were injected over 0.5 min into the inferior vena cava, followed by 2 mL of fetal blood. Reference blood samples were withdrawn from the axillary artery at a rate of 1.03 mL/min, beginning 30 s before the microsphere injection and continuing for 1.0 min after the injection was completed. After completion of the study, the ewe was killed with an overdose of pentobarbital sodium followed by saturated KCl solution. The fetus was removed and weighed, and then the brain was immediately removed at the base and divided at the cephalic border of the pons. All supratentorial tissue (including cerebral hemispheres and midbrain) was pooled and counted to determine CBF. The cerebellum was counted separately as was the brainstem (pons and medulla). The radioactivity in all samples was determined by using a multichannel gamma-counter (Packard Instrument, Downers Grove, IL). All reference and tissue samples contained >400 microspheres.

Blood samples for pH, respiratory blood gases, Hb concentration, and O_2 saturation were anaerobically withdrawn into heparinized Natelson glass pipettes. Respiratory blood gases and pH were measured at 39.5°C using the Radiometer ABL 30 (Radiometer, Copenhagen, Denmark). O_2 saturation and Hb concentration were measured with the OSM-3 Hemoximeter.

Arterial blood pressure (referenced to amniotic fluid pressure) and heart rate were continuously monitored (Gould Instruments, Oxnard, CA).

Whole blood (2 mL) for cocaine and its metabolites was collected in nonheparinized syringes and immediately placed into tubes containing 0.1 mL of enzyme inhibitor (equal parts of a saturated sodium fluoride solution and a 10% vol/vol solution of glacial acetic acid). The blood was mixed with the inhibitor, centrifuged, and the plasma stored frozen (−70°C) until analyzed.

Cocaine, ecgonine methyl ester, and benzoylecgonine were isolated using solid-phase extraction as described by Cone *et al.* (22). Extraction cartridges (Worldwide Monitoring Corporation, ZSDAU020) were conditioned sequentially with methanol, water, and pH 4 acetate buffer. Samples were diluted with deionized water and pH 4 acetate buffer, vortexed, centrifuged, and added to the cartridges. The cartridges were washed with water, 0.1 N HCl, and methanol. The cartridges were dried under vacuum and treated with the elution solvent (methylene chloride/isopropanol/ammonium hydroxide, 80:20:2% vol/vol). The extracts were evaporated under argon and reacted with bis(trimethylsilyl)trifluoracetamide + 1% trimethylchlorosilane in acetonitrile. The gas chromatography/mass spectrometry analyses were performed with an HP 5890A Series II gas chromatograph and 7673A automatic liquid sampler interfaced with an HP 5971A mass selective detector operated in the selected ion monitoring mode.

Experimental protocol. On the day of study, the ewe was brought into the laboratory and placed in a specially designed study cart with free access to food. The ewe was allowed at least 1 h to become accustomed to her surroundings. During this time, 10 mL of maternal blood was withdrawn from the femoral artery catheter and infused into the fetal inferior vena cava while 10 mL of fetal blood was slowly withdrawn from the fetal axillary artery. This exchange procedure provided blood for replacement during the study without significant changes in the fetal Po_2 at which Hb is one-half saturated with O_2 (P_{50}) which may be expected with direct transfusion of maternal blood. A total of 9 mL of fetal blood were withdrawn during the entire study, which is approximately 10% of fetal blood volume.

For each measurement, blood samples were slowly withdrawn from the fetal axillary artery and superior sagittal sinus, and the maternal femoral artery (0.3 mL each vessel) and analyzed for pH, Hb concentration, O_2 saturation, and blood gases. In addition, arterial blood (2 mL) was analyzed for cocaine and its metabolites in ewes before and at 5 and 15 min after cocaine injection, and in fetuses only at 5 min. After blood was sampled, radiolabeled microspheres (0.3 mL) were injected into the inferior vena cava while reference samples were withdrawn from the axillary artery. All withdrawn blood was immediately replaced with warmed fetal blood obtained from the exchange transfusion procedure.

One baseline measurement was obtained. Then, pure cocaine hydrochloride (Sigma Chemical Co., St. Louis, MO; 2 mg/kg

Table 1. *Systemic responses to 2 mg/kg maternal cocaine injection in immature fetal sheep*

	Baseline	Time after maternal cocaine injection (min) 2	5	15
MAP (mm Hg)	39 ± 1	43 ± 1	48 ± 2*	42 ± 2
Heart rate (beats/min)	206 ± 4	216 ± 6	225 ± 6	234 ± 9
Arterial pH	7.32 ± 0.02	7.32 ± 0.01	7.30 ± 0.02	7.30 ± 0.02
$Paco_2$ (kPa)	6.5 ± 0.13	6.5 ± 0.13	6.8 ± 0.13	6.7 ± 0.13
Pao_2 (kPa)	2.8 ± 0.13	2.8 ± 0.13	2.4 ± 0.27*	2.9 ± 0.13
Hematocrit, %	0.32 ± 0.01			0.31 ± 0.007
Hemoglobin (gm/L)	91 ± 2	94 ± 2	93 ± 3	92 ± 2
Arterial O_2 saturation, %	62 ± 2	50 ± 5*	49 ± 5*	57 ± 4

Values are means ± SEM; $n = 9$ fetal sheep.
* $p < 0.05$, compared with baseline.

maternal weight dissolved in 5 mL of 0.9% saline) was injected over 10 s into the maternal jugular vein followed by 5 mL of 0.9% saline flush. Measurements were made 2, 5, and 15 min after cocaine injection.

Data analysis and calculations. CBF was calculated as CBF = $(cpm_{brain}/cpm_{ref}) \times 1.03$ mL/min, where cpm_{brain} and cpm_{ref} represent radioactive counts/min in brain and reference samples, respectively. Other organ blood flows were calculated similarly. Cerebral metabolic rate of O_2 ($CMRo_2$) was calculated as $CMRo_2 = (Cao_2 - Cvo_2) \times$ CBF, where Cao_2 and Cvo_2 represent arterial and venous O_2 content, respectively. Cerebral O_2 delivery (OD) was calculated as $Cao_2 \times$ CBF and cerebral O_2 extraction (E) as $CMRo_2 \div$ OD. Vascular resistance was calculated as MAP per organ blood flow.

Measurements were calculated and data are reported as mean ± SEM for all study fetuses. Differences between groups were analyzed by repeated-measures analysis of variance. If the F test was significant, specific differences were sought with the Newman-Keuls test. Significance was considered at $p < 0.05$.

RESULTS

Fetal cardiovascular variables, hematocrit, and arterial blood gases at baseline and in response to maternal cocaine injection are shown in Table 1. Baseline values are consistent with previous physiologic studies in immature fetal sheep. There were no changes in fetal heart rate, arterial pH, $Paco_2$, Hb, or hematocrit during the study.

Fetal CBF, MAP, CVR and Cao_2 at baseline, and in response to maternal cocaine injection are depicted in Figure 1*a–d* and Tables 2 and 3. There was a 19 ± 9% increase in CBF (Fig. 1*a* and Table 2) at 5 min (64 ± 17 *versus* 52 ± 9 mL/100 g/min at baseline). Other regional brain blood flow responses (Table 2) paralleled the changes in CBF at 5 min with the greatest percentage increase in blood flow occurring in brainstem (↑ 31 ± 16%). Blood flow returned to baseline by 15 min in each region. Mean arterial blood pressure (Fig. 1*b*) increased (↑ 21 ± 6%) 5 min after maternal cocaine injection and returned to baseline by 15 min. Cerebrovascular resistance, calculated as CBF/MAP (Fig. 1*c* and Table 3), increased at 2 min and returned to baseline at 5 min. There were no changes in calculated vascular resistance in cerebellum or brainstem (Table 3). Cao_2 (Fig. 1*d*) decreased at 2 and 5 min, primarily because of a decrease in arterial O_2 saturation, as noted in Table 1. There was also a small but significant decrease in Pao_2 (↓ 16 ± 5%) at 5 min. Both Cao_2 and Pao_2 returned to baseline by 15 min.

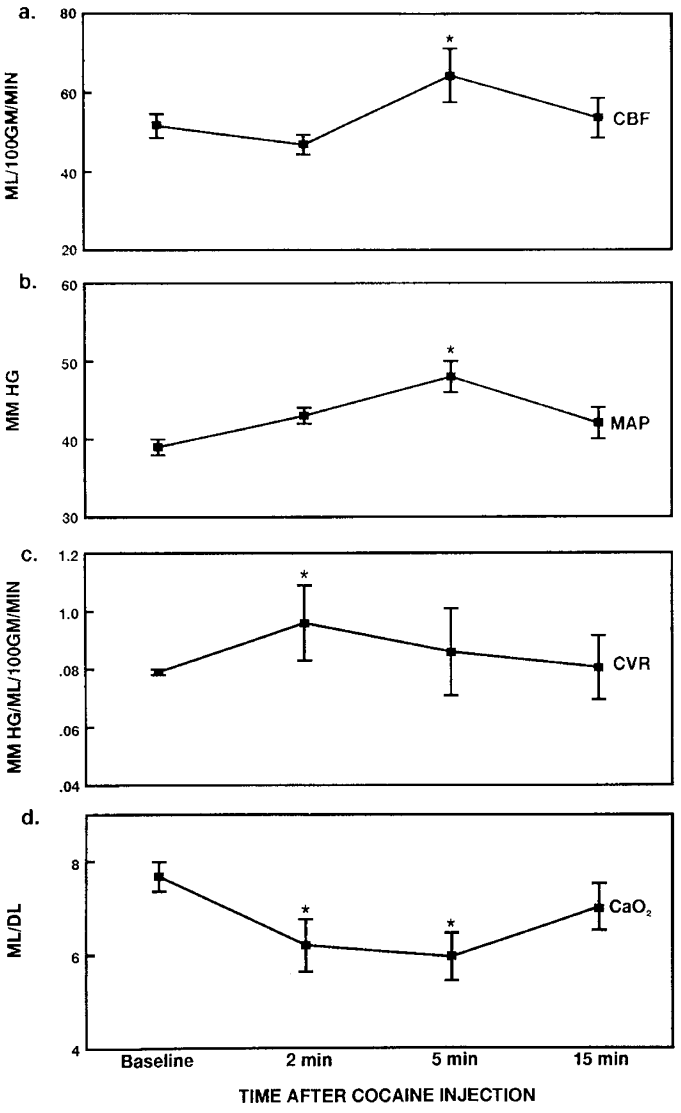

Figure 1. Fetal CBF (*a*), MAP (*b*), CVR (*c*), and Cao_2 (*d*) in nine immature fetal sheep (mean ± SEM) at baseline and at 2, 5, and 15 min after maternal cocaine injection (2 mg/kg) (1 mM O_2 = 22.4 mL). *$p < 0.05$ compared with baseline.

Cerebral O_2 metabolism data are shown in Table 4. In two of nine animals, there were technical problems with the sagittal

Table 2. *Regional brain blood flow (mL/100 g/min) responses to 2 mg/kg maternal cocaine injection in immature fetal sheep*

		Time after maternal cocaine injection (min)		
	Baseline	2	5	15
Cerebrum	52 ± 3	47 ± 3	64 ± 7*	54 ± 5
Cerebellum	81 ± 7	83 ± 8	102 ± 14*	94 ± 11
Brainstem	120 ± 9	117 ± 10	157 ± 20*	149 ± 16

Values are means ± SEM; $n = 9$ fetal sheep.
* $p < 0.05$, compared with baseline.

Table 3. *Regional vascular resistance (mm Hg/ml/100 g/min) in response to 2 mg/kg maternal cocaine injection in immature fetal sheep*

		Time after maternal cocaine injection (min)		
	Baseline	2	5	15
Cerebrum	0.79 ± 0.1	0.96 ± 0.13*	0.86 ± 0.15	0.81 ± 0.11
Cerebellum	0.48 ± 0.04	0.53 ± 0.06	0.52 ± 0.07	0.45 ± 0.05
Brainstem	0.34 ± 0.03	0.39 ± 0.03	0.34 ± 0.05	0.31 ± 0.03

Values are mean ± SEM; $n = 9$ fetal sheep.
* $p < 0.05$, compared with baseline.

Table 4. *Fetal cerebral O_2 metabolism before and after maternal cocaine injection*

		Time after maternal cocaine injection (min)		
	Baseline	2	5	15
Arterial O_2 content (mL/dL)	7.66 ± 0.32	6.19 ± 0.56*	5.94 ± 0.51*	7.01 ± 0.50
O_2 Consumption (mL/100 g/min)	1.19 ± 0.11	0.86 ± 0.13	1.20 ± 0.19	1.42 ± 0.15
O_2 delivery (mL/100 g/min)	3.72 ± 0.20	2.83 ± 0.24*	3.32 ± 0.26	3.60 ± 0.25
Fractional O_2 extraction	0.31 ± 0.02	0.34 ± 0.05	0.34 ± 0.04	0.37 ± 0.02

Values are means ± SEM; $n = 9$ fetal sheep for O_2 content and O_2 delivery and $n = 7$ for O_2 consumption and O_2 extraction; 1 mM O_2 = 22.4 mL.
* $p < 0.05$, compared with baseline.

sinus catheter; therefore, $n = 7$ for $CMRo_2$ and fractional O_2 extraction. Cerebral O_2 delivery decreased at 2 min, but returned to baseline by 5 min. There were no changes in $CMRo_2$ or fractional O_2 extraction throughout the study.

Arterial plasma cocaine and cocaine metabolites (maternal and fetal) are shown in Table 5. Fetal blood was analyzed only at 5 min. Cocaine levels at baseline were zero in maternal blood and were therefore assumed to be zero in fetal blood. At 5 min, cocaine levels were 226 ± 80 ng/mL (fetal) and 303 ± 267 ng/mL (maternal). Cocaine was rapidly metabolized to ecgonine methyl ester in both ewe and fetus, and ecgonine methyl ester levels remained elevated at 15 min. Maternal cocaine levels at 5 min are approximately 60% higher than human levels reported 30 min after a moderate (40 mg) cocaine dose (23).

Table 5. *Arterial cocaine and cocaine metabolite blood levels (ng/mL) in immature sheep and their mothers after maternal cocaine injection*

		Time after maternal cocaine Cocaine injection (min)	
	Baseline	5	15
Fetus			
Cocaine		225 ± 27	
Benzoylecgonine		144 ± 27	
Ecgonine methyl ester		488 ± 86	
Mother			
Cocaine	0	303 ± 89	131 ± 39
Benzoylecgonine	0	152 ± 17	182 ± 23
Ecgonine methyl ester	0	786 ± 61	732 ± 50

Values are mean ± SEM; $n = 7$ fetal sheep, $n = 9$ mothers.

DISCUSSION

The major findings of this study are that immature fetal sheep, like mature fetuses, respond to maternal cocaine injection with hypoxemia and hypertension. However, unlike mature fetuses, immature fetuses demonstrate cerebral vasoconstriction at 2 min with decreased cerebral O_2 delivery, and at 5 min no vasodilation is observed in any brain region.

Most studies of the fetal cerebral circulation have been performed in near-term fetal sheep, but the fetal sheep brain is quite mature near term, both structurally and functionally. The 90-d gestation sheep brain is comparable to a 26-wk gestation human brain (24–27), a gestational age at which approximately 90% of prematurely born infants in America now survive with excellent neonatal care (28, 29). It has thus become important to perform fetal cerebral studies in sheep earlier in gestation to make comparisons of results with other mammalian species. A growing body of literature now suggests that cerebrovascular responses of the developing sheep fetus are indeed uniquely different from responses of more mature fetuses. Immature fetal sheep at 90-d gestation have, for example, significantly lower CBF, $CMRo_2$, and CMRglu compared with mature fetal sheep (15, 40). Although CO_2 responses are normal (30), cerebral vasodilatory responses to hypoxemia are blunted (16), cerebral autoregulation in response to intracranial hypertension is poorly developed (31); and cerebrovascular and metabolic responses to acute maternal alcohol intoxication are virtually absent (32). It is therefore not surprising that immature fetal cerebrovascular responses to maternal cocaine would differ from more mature fetuses.

Fetal systemic and cerebrovascular responses to maternal cocaine injection have been well described in near-term pregnant sheep. Woods *et al.* (33) reported a 22% increase in fetal MAP, a 47% decrease in uterine blood flow and a 21% decrease in fetal arterial Po_2 5 min after a 2 mg/kg maternal cocaine injection. Moore *et al.* (34) noted fetal hypertension and decreased uterine blood flow after a 1 mg/kg injection of cocaine to pregnant ewes. We previously reported a 22% increase in fetal MAP, a 33% decrease in fetal Cao_2, and vasodilation in some brain regions 5 min after a 2 mg/kg maternal cocaine injection near term in sheep. We have also reported cerebral vasodilation after direct cocaine injection in mature fetal sheep and newborn lambs (11, 14). However, other investigators, using different species and methodology, have reported that cocaine constricts cerebral vessels (7–10). The results from the present study suggest that developmental differences in the cerebrovascular responses to cocaine may partially account for some of these conflicting results. More immature vessels, such as those of the 90-d sheep fetus, or the newborn rat or piglet, may respond to cocaine by vasoconstriction, whereas more mature vessels dilate.

The vasoconstriction we observed in all brain regions at 2 min differs significantly from our previous findings in mature fetuses in which we observed no changes in vascular resistance at 2 min. Similar increases in MAP and decreases in Cao_2 were observed, thus making it unlikely that differences in fetal systemic responses to maternal cocaine accounted for the different cerebrovascular responses. Furthermore, incomplete development of autoregulation in immature fetuses could not account for this difference because an increase in MAP with impaired autoregulation would have resulted in cerebral vasodilation, not vasoconstriction. Cocaine is a sympathomimetic drug that exerts its direct effects on peripheral blood vessels by enhancing responsivity to catecholamines (35), by blocking the reuptake of norepinephrine from perivascular nerve terminals (36) and by increasing catecholamine output from the adrenal medulla (37). Mechanisms for cocaine's cerebrovascular responses are less well understood, but may depend in part on the ability of cocaine and its metabolites, as well as norepinephrine, to cross the blood-brain barrier. The blood-brain barrier in fetal sheep at 90 d gestation is well developed (38). However, it is not known what effect hypertension and/or hypoxemia may have on its integrity in immature sheep. If it is disrupted, then one might observe increased brain cocaine and/or norepinephrine levels with consequent vasoconstriction. Alternatively, vascular responses in the immature fetus may reflect unopposed vasoconstriction due to limited vasodilatory capacity. We have previously shown that the CBF response to hypoxemia is attenuated in preterm fetal sheep (16). Since cocaine was associated with a similar decrease in Cao_2 at 2 min in mature and immature fetuses, we could speculate that inadequate release of O_2-sensitive vasoactive substances (such as nitric oxide or adenosine) occurred in preterm fetuses, allowing unopposed vasoconstrictive effects of cocaine or norepinephrine. In support of this hypothesis, Northington *et al.* (39) have recently shown that nitric oxide synthase activity in cortex increases to adult levels after 90-d gestation in fetal sheep.

The transient cerebral vasoconstriction at 2 min could potentially have an adverse effect on the developing brain, particularly if maternal cocaine use occurs in a chronic, repetitive fashion. Cerebral O_2 delivery was decreased, albeit for a short period of time, and this could make certain brain regions more vulnerable to hypoxic-ischemic brain injury. Although we were only able to measure O_2 consumption by cerebrum (and noted no change), other brain regions could have increased their O_2 consumption in response to cocaine or catecholamines and could therefore become vulnerable to decreases in O_2 delivery, particularly if such regions are unable to increase their O_2 extraction.

The lack of cocaine-induced cerebral vasodilation at 5 min (compared with baseline, Fig. 1*c*) also contrasts with our previous findings in mature fetal sheep in which we observed vasodilation in some brain regions. Of note, however, is that although there was no decrease in CVR at 5 min compared with baseline, there was a decrease in CVR from the elevated 2-min measurement (Fig. 1*c*), suggesting either that cocaine actually had limited vasodilatory effects in immature fetuses or that its vasoconstrictive effect was very transient. In our previous study in near-term fetuses, we were unable to explain cocaine-induced cerebral vasodilation on the basis of coupling to an increase in $CMRo_2$ (there was none) nor solely as a response to hypoxemia. We speculated that it was likely due to the combined effects of fetal cerebral responses to hypoxemia, hypertension (with impaired autoregulation), and to direct or indirect effects of cocaine or its metabolites on fetal cerebral vessels. A subsequent study in which we injected cocaine directly to the fetus supported the hypothesis that cerebral vasodilation is not due solely to fetal hypoxemia but rather is largely a direct or indirect effect of cocaine or its metabolites on cerebral blood vessels, independent of the uteroplacental circulation (14). Because we observed less hypoxemia at 5 min in the immature fetuses than we had observed in the mature fetuses, this could explain in part the absence of cerebral vasodilation at 5 min (compared with baseline). Less significant hypoxemia in the immature fetuses could have occurred because of the multicotyledonary sheep placenta which has a larger surface area relative to fetal size and blood volume earlier in gestation and consequently may render the preterm fetus potentially less vulnerable to uteroplacental vasoconstriction from maternal cocaine than the term fetus, with consequently less fetal hypoxemia. Another explanation for the absence of cocaine-induced vasodilation is the blunted vasodilatory response to hypoxemia by immature fetuses which we have previously reported, resulting in decreased O_2 delivery. This is not due to undeveloped vasoreactivity to adenosine (41), but rather reflects either immature regulatory mechanisms or an inability of cerebral vessels to respond to the usual stimuli (16). Finally, an increase in fetal P_{50} secondary to maternal blood transfusion could have altered cerebrovascular responses to cocaine and/or to hypoxemia. However, our method of maternal-fetal exchange transfusion before study to provide blood for replacement was successful in preventing any changes in fetal P_{50} (estimated using oxygen saturations, a Hill coefficient of 2.58 and a Bohr factor of 0.44 corrected to pH 7.40). Therefore, alterations in fetal oxygen affinity could not explain the absence of cocaine-induced cerebral vasodilation.

Although we did not observe cerebral vasodilation at 5 min in immature fetuses, CBF increased enough to maintain cerebral O_2 delivery, unlike the response at 2 min. However, an acute increase in CBF may be detrimental to immature brain, particularly when accompanied by acute changes in blood pressure. In preterm infants, acute increases in blood pressure, abrupt changes in cerebral blood flow velocity and acute volume expansion are associated with intracranial hemorrhages (42, 43).

Fetal and maternal cocaine levels were similar to those reported in our previous studies using a 2 mg/kg maternal cocaine injection near-term (12). We were limited to a single fetal cocaine level because of the volume of blood required for an accurate measurement (2 mL) and the limitations in donor fetal blood that we had. We chose a fetal measurement at 5 min because that is the time at which the most significant fetal responses were seen in our near-term fetal studies. There were no significant differences between fetal and maternal cocaine levels at 5 min, nor were there differences noted in cocaine levels between the present study and our previous study in mature fetuses. Maternal cocaine levels are presumably higher before 5 min as there was evidence of rapid metabolism to ecgonine methyl ester by 5 min. Metabolism of cocaine in sheep is different from in humans; it is more rapid, and ecgonine methyl ester is a major sheep metabolite whereas benzoylecgonine, a major human metabolite, is not.

In summary, immature fetuses respond differently to maternal cocaine injection than mature fetuses because despite transient hypoxemia, they demonstrate initial cerebral vasoconstriction, with decreased cerebral O_2 delivery, and no subsequent cerebral vasodilation. Developmental differences in fetal responses to maternal cocaine may be due to differences in integrity of the blood-brain barrier, different levels of or responses to hypoxemia or to differences in vascular responses to cocaine, its metabolites, or to norepinephrine.

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