Chronic Prenatal Exposure to Cocaine Alters Cerebrovascular Responses in Newborn Pigs

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Maternal cocaine abuse may increase the incidence of perinatal asphyxia. In nonexposed asphyxiated neonates, decreased cerebrospinal fluid (CSF) cAMP concentrations are associated with poor neurological outcome. On the other hand, cocaine increases central nervous system (CNS) cAMP. Therefore, we hypothesized that *in utero* cocaine exposure may increase brain cAMP and thereby preserve cerebrovascular responses to cAMP-dependent stimuli following asphyxia. Pregnant pigs received either cocaine (1 mg/kg, iv) twice weekly during the last trimester or normal saline vehicle (sham-control) and were allowed to deliver vaginally at term. Cranial windows were implanted in the newborn pigs within the first week of life and used to collect CSF for cAMP determinations and to assess changes in pial arteriolar diameters (PAD). In the first part of the study, pial arteriolar responses to different vasodilator and vasoconstrictor stimuli were evaluated in piglets prior to asphyxia (n = 20). In newborn pigs exposed to cocaine, cerebrovascular responses to hypercapnia and norepinephrine were significantly exaggerated compared to controls. Then, piglets were randomly selected for the second part of the study that involved prolonged asphyxia (n = 12). In cocaine-exposed but not sham-control piglets, CSF cAMP increased markedly during asphyxia. In the sham piglets, but not the cocaine-exposed piglets, CSF cAMP fell progressively below the baseline during recovery. Cerebrovascular reactivity to cAMP-dependent stimuli (hypercapnia and isoproterenol) was preserved during recovery from asphyxia in the cocaine-exposed piglets but significantly attenuated in the sham controls. We conclude that piglets with chronic prenatal exposure to cocaine show exaggerated cerebrovascular responses to vasogenic stimuli and preserved cAMP-dependant cerebral vasoreactivity following asphyxia. Exp Biol Med 229:819–825, 2004

Key words: cocaine; cAMP; asphyxia; pial arterioles; neonates

Introduction

In utero exposure to illicit drugs, including cocaine, remains a significant public health problem (1, 2). However, reports on neonatal neurological outcome associated with maternal cocaine abuse are conflicting (3–5). Cocaine interferes with presynaptic catecholamine reuptake and transport, which results in catecholamine accumulation postsynaptically (6). Catecholamines such as epinephrine and dopamine activate adenylyl cyclase, increasing intracellular cAMP. Cyclic AMP participates in intracellular events by acting as a second messenger, and many cerebral vasodilatory responses are mediated by cAMP. There are only limited reports on the effects of antenatal cocaine exposure on neonatal cerebral circulation.

Maternal cocaine abuse may increase the incidence of perinatal asphyxia (7–8). Severe asphyxia causes decreases in cerebrospinal fluid (CSF) cAMP that correlate with the degree of asphyxia. Low brain cAMP is associated with altered cerebral vascular reactivity, brain metabolism, and energy state (9–11). Among newborn infants who suffered prenatal asphyxia, those infants whose mothers abused cocaine during pregnancy had CSF cAMP within normal ranges, whereas those whose mothers did not use cocaine had reduced CSF cAMP (12). Furthermore, asphyxiated infants with normal cAMP had better neurological outcomes at 12 months of age than those with reduced values (12).

Thus, we hypothesized that neonatal pigs exposed to cocaine *in utero* may experience less cerebrovascular

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derangement from chronic asphyxia than those with no cocaine exposure.

Materials and Methods

The animal protocol was reviewed and approved by the Animal Care and Use Committee of the University of Tennessee Health Science Center.

Three 4-month-old pigs were impregnated. During the last trimester of pregnancy, two pigs received cocaine, 1 mg/kg, iv, twice weekly through an ear vein. The other pig (sham control) received normal saline instead of cocaine. At this dose, the blood concentrations of cocaine and its metabolites in pigs are similar to those of drug-abusing adult humans (13, 14), and pregnant nonhuman primates tolerate 1 mg/kg, iv, cocaine without experiencing seizures or fetal losses (15). The pregnant pigs had access to food and water *ad libitum*. The pregnancies were uneventful; all 3 pigs delivered vaginally at term about 2 days after the last dose of cocaine/saline. The piglets' birth weights were comparable between the cocaine and the sham-control groups.

During the next 10 postnatal days, the effects of vasogenic stimuli on cerebrovascular reactivity and also the effects of prolonged asphyxia on cerebral microcirculation were evaluated in both cocaine-exposed and sham-control (saline-treated) newborn piglets (cocaine, $n = 11$; sham control, $n = 9$). Cocaine and sham-control piglets were evaluated in alternating order.

Catheter Placement. Piglets 3 to 10 days of age (1.6 to 4.1 kg) were anesthetized with a mixture of ketamine hydrochloride (33 mg/kg, im) and acepromazine (3.3 mg/kg, im) and maintained on α-chloralose (30 mg/kg, iv, followed by 3 mg/kg every 3 hrs). The femoral artery and vein were cannulated. The arterial catheter was used to monitor blood pressure and to withdraw blood samples for blood gases and pH analysis; the venous catheter was used to administer anesthesia and fluid (D_5W at 4 $ml \cdot kg^{-1} \cdot hr^{-1}$). The trachea was cannulated, and the piglets were ventilated mechanically with room air using a newborn positive pressure respirator (Bournes BP-200). The systemic arterial blood pressure (BP), pH, and gases were maintained within the normal range during the experiments prior to induction of asphyxia. All piglets were paralyzed with pancuronium bromide during asphyxia. Body temperature was maintained between 37°C and 38°C with a heating pad that was connected to a servo-control system.

Closed Cranial Window Placement. Closed cranial windows were implanted over the left parietal cortices for measurements of pial arteriolar diameters (PADs), topical application of vasodilator and constrictor stimuli, and collection of cortical periarachnoid CSF. To implant a closed cranial window, the head was immobilized, and the scalp over the left parietal cortex was cut and retracted. A hole 2-cm in diameter was made in the skull, and the dura was retracted. A stainless steel ring with a premounted glass pane was inserted in the hole. The window was cemented in place with dental acrylic. Three needles piercing the ring allowed injection of artificial CSF (aCSF) under the window and sampling of CSF. The space under the window (500 μl) was filled with aCSF composed of (m*M*) 3.0 KCl, 1.5 $MgCl_2$, 1.5 $CaCl_2$, 132 NaCl, 6.6 urea, 3.7 dextrose, and 24.6 $NaHCO_3$; approximate pH of 7.33, PCO_2 of 45 mm Hg, and PO_2 of 42 mm Hg. Pial arterioles were observed with a dissecting microscope, a television camera mounted on the microscope, and a video monitor. A video micrometer was used to measure PAD.

Materials. Cocaine was purchased from RBI (Natic, MA). Pancuronium bromide was purchased from Astra Pharmaceutical Products Inc. (Westborough, MA). All other materials were purchased from Sigma Chemical Company (St. Louis, MO).

Experimental Procedures. Thirty mins after confirming that arterial blood pressure, pH, and blood gases were within the normal range, 2 to 4 pial arterioles of 60–120 μm in diameter were chosen in each piglet. Changes in diameters of pial arterioles were recorded throughout the experiments. The average of the pial arteriolar diameters in each piglet was used as the data point for statistical analysis.

Arterial blood was collected periodically for blood gases and pH measurement using a Blood Gas Analyzer (Instrumentation Laboratory, Lexington, MA). A total volume of 2 to 4 ml blood was drawn from each piglet for measurements of arterial blood pH and gases. These piglets did not require blood transfusion because the amount of blood withdrawn was much less than 10% of the total blood volume. Blood pressure and heart rate were monitored continuously during the experiments. CSF was collected from a cisternal tap in each piglet only at the start of the experiment. However, cortical periarachnoid CSF was collected frequently during baseline, during vasogenic stimulation, and also during asphyxia and recovery for later measurements of cAMP. The periarachnoid CSF was undisturbed for 10 mins prior to collection of the CSF samples. At the end of 10 mins, the space under the cranial window was flushed gently and repeatedly with aCSF prior to the next challenge.

First, newborn pigs were evaluated for cerebrovascular responses to vasodilator and vasoconstrictor stimuli in a random order: hypercapnia ($PaCO_2$, 60 to 70 mm Hg), hypoxia (PaO_2, 18 to 20 mm Hg), isoproterenol (10^{-4} *M*), sodium nitroprusside (10^{-4} *M*), hypocapnia ($PaCO_2$, 15 to 20 mm Hg), and norepinephrine (10^{-4} *M*). Maximal pial arteriolar responses to each vasogenic stimulus were recorded during a 10-min period.

After completing the cerebrovascular reactivity part of the study, some piglets were randomly assigned to asphyxia. Asphyxia was induced for 60 mins by ventilating the piglets with a gas mixture of 10% CO_2, 10% O_2, and 80% N_2 coupled with a decrease in the minute volume. Respiratory support and the gas mixture were manipulated to cause asphyxia for 60 mins while also maintaining systemic BP above 28 mm Hg and/or heart rate above 50 beats/min. At

Table 1. Arterial BP, pH, and Gases at Baseline, Asphyxia, and Recovery Periods in the Control and the Cocaine Groups[a]

Variables	Group	Baseline	Asphyxia	Recovery
MABP[b] (mm Hg)	Control	44 ± 4	29 ± 4**	41 ± 4
	Cocaine	56 ± 3*	28 ± 3**	44 ± 5
pH	Control	7.38 ± 0.02	6.94 ± 0.02**	7.20 ± 0.01
	Cocaine	7.37 ± 0.01	7.00 ± 0.02**	7.27 ± 0.01
PO_2 (mm Hg)	Control	95 ± 3	42 ± 2**	92 ± 2
	Cocaine	99 ± 14	43 ± 2**	106 ± 10
PCO_2 (mm Hg)	Control	35 ± 1	79 ± 2**	37 ± 1
	Cocaine	32 ± 2	68 ± 3**	31 ± 2

[a] Values are means ± SEM, *$P < 0.05$ cocaine compared to control, **$P < 0.05$ asphyxia compared to other periods. $n = 7$ control piglets; $n = 5$ cocaine piglets.
[b] Mean atrial blood pressure.

the end of 60 mins of asphyxia, the minute volume was increased, and the piglets were ventilated with room air. Piglets were evaluated continuously during asphyxia and recovery.

During the recovery from asphyxia, viability of cerebral microcirculation was investigated. Hypercapnia and isoproterenol were chosen because their vasodilatory effects are via cAMP-dependent mechanisms (16, 17). Vascular reactivities to hypercapnia ($PaCO_2$ >60 mm Hg) and to topically applied isoproterenol (10^{-4} *M*) were evaluated during 60 to 90 mins of recovery.

Cyclic AMP Analysis. The collected CSF (0.4 ml) was immediately mixed with EDTA (5 μl) and stored at −60°C until assayed. Cyclic AMP was measured by radioimmunoassay (RIA) procedures (10).

Statistical Analysis. The results are presented as means ± SE. Comparisons among different time periods were made using analysis of variance with repeated measures followed by the Fisher protected least significant difference test. Comparisons between the two groups were made using a *t* test for planned comparisons (paired or unpaired, as appropriate). $P < 0.05$ was selected as significant.

Results

Experiments were started when heart rate, arterial BP, pH, and blood gas values were within the normal range. Both groups started with a comparable heart rate (209 ± 20 vs. 209 ± 22 bpm). However, BP was significantly higher in the cocaine group compared to the control group (Table 1).

In the first part of the study, the cerebrovascular responses to hypercapnia and norepinephrine were significantly exaggerated in the cocaine-exposed group (Table 2). Although the differences did not reach statistical significance in the individual comparisons, dilations to hypoxia, isoproterenol, and sodium nitroprusside and constriction to hypocapnia were overall also accentuated in the cocaine-exposed piglets compared to the controls.

The severity of asphyxia was similar in both groups (Table 1). The BP decreased to similar levels during asphyxia in both groups and recovered to about the same BP upon reventilation. During recovery, piglets were ventilated with room air, and no attempt was made to correct the metabolic acidosis or the persistent systemic hypotension that followed prolonged asphyxia.

The concentration of cisternal CSF cAMP at the start of the experiments was not significantly different between the two groups (40 ± 7 and 34 ± 5 pmol/ml in the cocaine-exposed and the controls, respectively; $P = 0.2$). In the periarachnoid CSF, the cAMP concentration in the cocaine group was more than 2-fold higher than in the sham-control group (800 ± 200 and 300 ± 100 fmol/ml in the cocaine and the control group, respectively; $P = 0.06$). During late asphyxia, the cAMP level was up to 2-fold higher than the baseline in the cocaine group (Fig. 1) but not significantly changed in controls. During the recovery, cAMP declined in both groups, but in the control group, cAMP fell progressively below the baseline level throughout recovery, whereas in the cocaine-exposed piglets, cAMP was at or near baseline through 50 mins of recovery.

With the onset of asphyxia, significant pial arteriolar dilatation occurred in both groups (Fig. 2). However, pial

Table 2. Pial Arteriolar Responses to Vasodilator and Vasoconstrictor Stimuli (as % Change) in the Control and the Cocaine Groups[a]

Group	Hypercapnia	Hypocapnia	Hypoxia	Isoproterenol	Norepinephrine	Sodium nitroprusside
Control	46 ± 5	−10 ± 1	31 ± 2	38 ± 6	−33 ± 5	49 ± 7
Cocaine	60 ± 6*	−13 ± 2	37 ± 4	42 ± 4	−45 ± 3*	53 ± 4

[a] Values are means ± SEM, *$P < 0.05$ cocaine compared to control. $n = 9$ control piglets; $n = 11$ cocaine piglets.

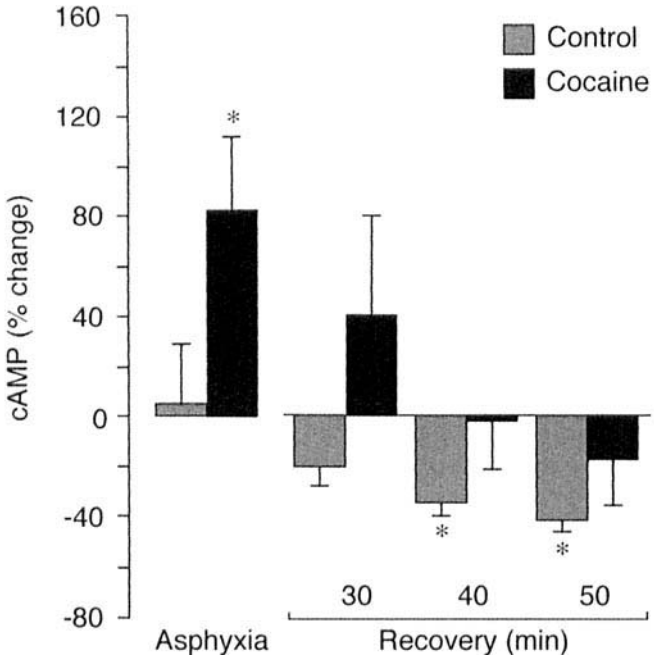

Figure 1. Percent change in CSF cAMP during late asphyxia and recovery in newborn pigs. Control $n = 7$; cocaine $n = 5$. Values are means ± SEM. $^*P < 0.05$ compared to baseline.

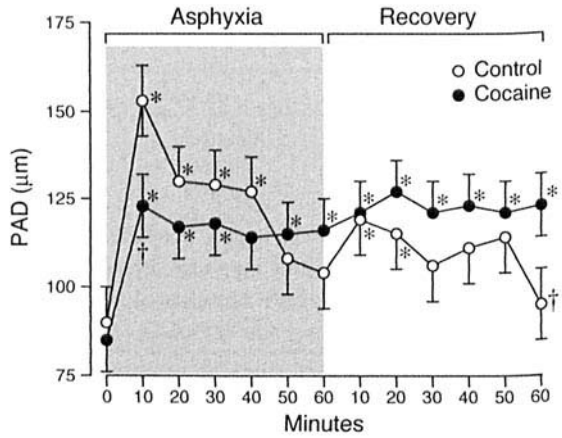

Figure 2. Changes in pial arteriolar diameters during asphyxia and recovery in newborn pigs. Control $n = 7$; cocaine $n = 5$. Values are means ± SEM. $^*P < 0.05$ compared to baseline. $^+P < 0.05$ cocaine compared to control.

arteriolar dilation was more pronounced during the early phase of asphyxia in the control group compared to the cocaine group. In the cocaine group, pial arterioles remained dilated during asphyxia and recovery. In the control group, PAD eventually decreased to nearly the baseline level by 60 mins of recovery.

In the sham-control group, cerebrovascular responses to both hypercapnia and isoproterenol were significantly ($P < 0.05$) decreased during recovery from asphyxia when compared with before asphyxia (Fig. 3). Conversely, in the cocaine group, cerebrovascular dilations to both hypercapnia and isoproterenol were preserved during recovery from asphyxia compared to before asphyxia (Fig. 3).

Discussion

The new findings are as follows: newborn pigs exposed to cocaine during the third trimester of pregnancy have higher brain cAMP levels, exaggerated cerebrovascular responses to vasodilator and vasoconstrictor stimuli, and better cerebral vascular reactivity during recovery from postnatal asphyxia than nonexposed piglets.

Conflicting reports exist on the effects of maternal cocaine abuse on their neonates (7, 18, 19). Cocaine readily crosses the placenta and also passes the blood-brain barrier and accumulates in the fetal CNS (20, 21). Cocaine is a unique and complex drug with sympathomimetic, local anesthetic, serotoninergic, and glutamatergic properties that all together may result in a wide range of cardiovascular, neural, and behavioral effects (7). The major effect of cocaine on CNS is inhibition of monoamine reuptake with postsynaptic accumulation of the neurotransmitters. Persistent increases in the concentration of monoamines can result in altered receptor sensitivity and/or density (22), which may lead to modifications of pial arteriolar reactivities. Yakubu *et al.* (23) observed increased cerebral vasoconstrictive responses to endothelin-1 in cocaine-exposed piglets compared to nonexposed. We found enhanced cerebrovascular constriction in response to norepinephrine in cocaine-exposed piglets. Similar to our findings, Seidler and Slotkin (24) reported a selective noradrenergic synaptic hyperactivity in fetal rat brain after chronic exposure to cocaine. Upregulation of noradrenergic receptor sites was found with prolonged prenatal exposure to cocaine (22). Also, cocaine sensitizes blood vessels to the contractile action of norepinephrine via blockage of monoamine reuptake (25). The above mechanisms may be the reason for exaggerated cerebrovascular reactivity to norepinephrine. Furthermore, we observed exaggerated cerebrovascular reactivity to hypercapnia. During the perinatal period, hypercapnia-induced cerebral vasodilation is by a prostanoid and cAMP-dependant mechanism (26). Cocaine increases vascular endothelial production of prostaglandin E_2 (27), a cAMP-dependent vasodilator (28). In addition, cocaine upregulates cAMP pathways (29). Therefore, exaggerated vasodilatory responses to hypercapnia in our cocaine-exposed piglets could be explained by the above mechanisms. Although cerebrovascular responses to other stimuli were not different from the controls, a trend toward hyper-reactivity was present in the cocaine group. Exaggerated cerebral arteriolar responses (vasodilation or vasoconstriction) may cause fetal and neonatal brain to be prone to hemorrhagic and/or ischemic insults.

Although cocaine is a potent peripheral vasoconstrictor, reports of its effects on cerebral microcirculation have not been consistent, especially in fetuses and newborns. Effects of cocaine on cerebral blood flow (CBF) and PAD may vary based on species, age, dosage, and duration of cocaine, route of administration, and concomitant use of anesthesia.

Responses differed when cocaine was given to the pregnant subject *versus* directly to the fetus (30, 31). In unanesthetized fetal sheep, a single maternal cocaine injection resulted in increased (32), decreased (33), or unchanged (34) fetal CBF in different studies. In this study, we administered low-dose cocaine with modest frequency during the third trimester of pregnancy. Our finding is just a representative of this specific situation. Whether higher doses and/or more frequent dosing of cocaine administered throughout the pregnancy would change the results is unknown. Also, our study cannot predict the possibility of protective effects of cocaine during asphyxia in older piglets. However, based on our clinical findings, asphyxiated infants of mothers with a history of heavy drug abuse had normal cAMP in CSF and also normal neurological outcome at 12 months of age when compared with asphyxiated infants of mothers who did not use cocaine (12).

Though the vasoconstrictor effect of cocaine is thought to be sympathetically driven, the vasodilatory property of cocaine appears to be cAMP mediated. Cocaine upregulates the cAMP pathway (28). Cocaine may increase CNS cAMP through several mechanisms. Cocaine upregulates catecholamines (6). Epinephrine stimulates adenylyl cyclase by activating β-adrenergic receptors (30), which in turn induces cAMP-mediated vasodilation. Also, cocaine may have direct β-adrenergic receptor activity (35). Cocaine-induced cerebral vasodilation is inhibited by propranolol, a β-adrenergic receptor blocker, in newborn sheep and cats (35, 36). Cocaine also increases the CNS concentration of dopamine. Dopamine, by acting at the D-1 receptor site, activates adenylyl cyclase, thus increasing cAMP (37). Finally, cocaine can increase vascular endothelial production of prostaglandin E_2 (26), a cAMP-dependent vasodilator (27).

In unanesthetized fetal sheep, with chronic cocaine exposure throughout mid- and third-trimester of pregnancy, no cerebral vasoconstrictive effect was observed, and the fetuses showed brain-sparing effect to moderately acute hypoxia (38, 39). To the best of our knowledge, ours is the first study on the impact of chronic *in utero* cocaine on cerebral circulation of neonates with prolonged asphyxia. Pial arteriolar responses during asphyxia and recovery were different in our cocaine and saline groups. In the cocaine group, pial arterioles remained dilated during asphyxia and recovery period, though in the control group, PAD returned to baseline by 60 mins of recovery. Also, the cocaine-exposed piglets had preserved cerebrovascular reactivity following asphyxia when compared to the sham-control piglets.

The mechanisms involved in cerebrovascular protection during asphyxia in our cocaine-exposed piglets include multiple possibilities. Chronic maternal cocaine abuse may cause repetitive metabolic stress in the fetuses. This process may result in neuroprotection by "preconditioning" of the fetal brain. Potential mechanisms involved include induction of protective gene expression such as c-fos and heat

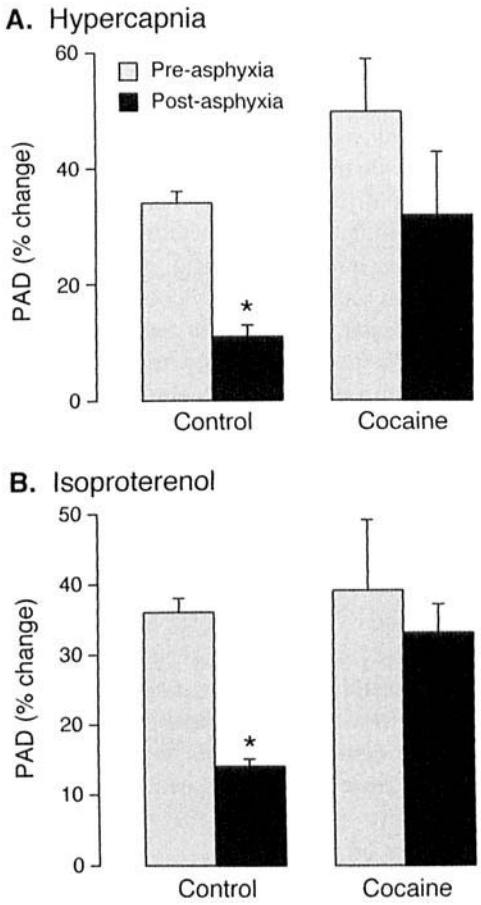

Figure 3. Cerebrovascular reactivity to (A) hypercapnia and (B) isoproterenol pre-asphyxia and post-asphyxia in newborn pigs. Control $n = 7$; cocaine $n = 5$. Values are means ± SEM. $^*P < 0.05$ compared to before asphyxia.

shock proteins (HSP70), upregulation of adenosine-A_1 type receptors, permanent neuronal GABA elevation, permanent K^+ channel activation, and enhancement of respiratory enzyme activity such as cytochrome-*c* oxidase, ATP-synthase, and succinate dehydrogenase (40). Therefore, in the the fetus that is chronically exposed to cocaine, the brain might get protection against future asphyxia insults by "preconditioning."

Also, cocaine is reported to have hypothermic effects in different species such as rats, mice, chicks, and guinea pigs, but not in rabbits (41). Hypothermia may protect the brain from oxidative stress–induced cellular injury and programmed cell death by increasing the activity of glutathione peroxidase activity and alterations in the expression of antiapoptotic protein, bcl-2 (42). We did not measure the body temperature in our pregnant pigs. However, our piglets were not hypothermic, and we maintained the temperature during the experiments by servo-control system.

Another possible mechanism for preservation of cerebrovascular reactivity in our asphyxiated cocaine piglets might be cocaine-induced upregulation of noradrenergic neurotransmitters, because studies have shown beneficial effects of noradrenergic neurotransmitter after brain injury

or cerebral ischemia (43, 44). Another possibility is the local anesthetic action of cocaine. Sodium ion entry into the nerve cells is controlled by voltage-gated sodium ion channels, which regulate nerve conduction. Blockade of the channels by cocaine and its metabolites would diminish nerve impulses (6). A common mechanism of modulation of Na-channel activity in brain and vascular tissue is via cAMP. cAMP reduces sodium influx in neurons, decreasing sodium channel number and activity and attenuating excessive harmful sodium currents (45). Cocaine blocks sodium channels and decreases release of excitatory amino acids, making neurons less susceptible to elevated excitatory amino acids and sodium and calcium ions that occur after asphyxia (46). Such an action may protect the brain from ischemic insults. In fact, acute administration of cariporide mesilate, a Na-channel blocker, caused preservation of cerebral vascular reactivity and CSF cAMP postasphyxia in piglets, similar to chronic cocaine exposure in the current experiments (47).

We conclude that neonatal pigs exposed to chronic *in utero* cocaine may experience accentuated cerebrovascular responses that could contribute to development of cerebral pathologies. However, these neonates may experience less cerebrovascular derangement from superimposed asphyxia compared to those with no cocaine exposure. Such "protection" may result from the tendency of cocaine toward upregulation of cAMP.

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