Prenatal Cocaine Exposure Causes Sex-Dependent Impairment in the Myogenic Reactivity of Coronary Arteries in Adult Offspring

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Abstract—Cocaine abuse is a significant problem among pregnant women. The present study tested the hypothesis that prenatal cocaine exposure impairs myogenic reactivity of coronary arteries in adult offspring. Pregnant rats received cocaine (30 mg kg\textsuperscript{-1} day\textsuperscript{-1}) or saline from days 15 to 21 of gestational age, and experiments were conducted in 3-month-old offspring. In pressurized coronary septal arteries, the diameter and vessel wall intracellular Ca\textsuperscript{2+} concentrations were measured simultaneously in the same tissue as a function of intraluminal pressure. Cocaine did not affect KCl-induced contractions of coronary arteries in either males or females but decreased the distensibility in male vessels. In male offspring, cocaine treatment resulted in a significant decrease in pressure-dependent myogenic contractions. Inhibition of eNOS with N\textsuperscript{G}-nitro-L-arginine did not alter the myogenic response in either saline control or cocaine-treated animals. In females, cocaine caused a significant increase in pressure-dependent myogenic contractions. N\textsuperscript{G}-nitro-L-arginine did not affect the myogenic response in the control animals but blocked the cocaine-mediated effect. In both males and females, the pressure-induced increases in vessel wall Ca\textsuperscript{2+} concentrations were not significantly different between cocaine and saline groups. The ratio of changes in the diameter to Ca\textsuperscript{2+} concentrations in the pressurized arteries was significantly less in male but greater in female offspring after cocaine treatment. The results suggest that prenatal cocaine exposure causes reprogramming of coronary myogenic tone via changes in the Ca\textsuperscript{2+} sensitivity in a sex-dependent manner, leading to an increased risk of dysfunction of coronary autoregulation in adult offspring. (Hypertension. 2009;54:1123-1128.)

Key Words: cocaine ■ fetal programming ■ gender ■ coronary artery ■ myogenic tone

Maternal cocaine abuse during pregnancy has been associated with numerous adverse perinatal outcomes. In addition to neurobehavioral effects, in utero cocaine exposure predisposes the fetus and neonate to various cardiovascular dysfunctions. Offspring born to mothers with a history of cocaine abuse have a high incidence of congenital cardiovascular malformations.\textsuperscript{1} It has been demonstrated in a rat model that fetal exposure to cocaine during gestation causes an increase in heart susceptibility to ischemia and reperfusion injury in adult male offspring.\textsuperscript{2} In addition, prenatal cocaine resulted in programming of enhanced vascular contractility via changes in eNOS-regulated Ca\textsuperscript{2+} sensitivity of myofilaments in sex- and tissue-dependent manners in resistance arteries, leading to an increased risk of hypertension in male offspring.\textsuperscript{3}

Consistently, epidemiological studies have shown a clear association of adverse intrauterine environment and an increased risk of coronary heart disease in adult life.\textsuperscript{4,5} However, it is unknown whether or to what extent fetal cocaine exposure affects coronary vascular reactivity in adult offspring. Coronary perfusion is essential for cardiac function, and autoregulation is an intrinsic tendency of coronary vasculature to maintain constant blood flow despite changes in perfusion pressure. Given that myogenic response is a major contributor to the autoregulation of coronary blood flow, the present study was designed to test the hypothesis that maternal cocaine administration during pregnancy alters coronary myogenic reactivity in adult offspring, which may lead to an increased risk of coronary heart disease in later life. The studies were performed in both male and female rat offspring to investigate the potential sex effects in fetal programming of the coronary reactivity caused by cocaine.

Methods

Experimental Animals

Time-dated pregnant Sprague-Dawley rats were purchased from Charles River Laboratories (Portage, Mich), and were randomly divided into 2 groups: (1) saline control, and (2) cocaine 15 mg/kg i.p. twice daily from day 15 to 21 of gestational age, as described previously.\textsuperscript{3} Offspring were studied at 3 months of age. All proce-
dures and protocols were approved by the Institutional Animal Care and Use Committee guidelines.

Coronary Artery Isolation and Cannulation
Hearts were isolated and the right ventricle was opened along the free wall. Septal coronary arteries were chosen to study intramural coronary arteries. The arteries (~100 to 150 μm in diameter and 2 to 3 mm in length) were isolated and cannulated in an organ chamber (Living Systems), followed by placement on the stage of an inverted microscope. The proximal cannula was connected to a pressure transducer and reservoir of physiological salt solution (PSS). The intraluminal pressure was controlled by a servo-system to set transmural pressures. The distal cannula was connected to a luer-lock valve that was open to flush the lumen during the initial cannulation. After cannulation, the valve was closed and measurements were conducted under no-flow conditions. Arterial diameter was recorded using the SoftEdge Acquisition Subsystem (IonOptix) as described previously.

Measurement of Myogenic Tone
Arteries were equilibrated in PSS for 10 minutes at intraluminal pressure of 20 mm Hg, after which pressure was increased from 20 to 70 mm Hg and returned to 20 mm Hg immediately. The vessels were then allowed to equilibrate at 20 mm Hg for another 30 minutes. After equilibration, intraluminal pressure was reduced to 10 mm Hg, and arteries were further stabilized for 10 minutes. The pressure was then increased in a stepwise manner from 10 to 100 mm Hg in 20-mm Hg increments, and each pressure level was maintained for 5 to 10 minutes to allow vessel diameter to stabilize before measurement. The passive pressure-diameter relationship was conducted in Ca²⁺-free PSS containing 3 mmol/L EGTA. Percent myogenic tone at each pressure step was calculated as % myogenic tone (D₁ - Dᵢ)/Dᵢ × 100, in which Dᵢ was the passive diameter in Ca²⁺-free PSS and Dᵢ was the active diameter with normal PSS in the presence of extracellular Ca²⁺.

Measurement of Vascular Wall [Ca²⁺]i
Intracellular free Ca²⁺ concentrations ([Ca²⁺]ᵢ) were measured in vascular wall loaded with fura-2 as previously described. Arterial segments were cannulated and pressurized to 10 mm Hg and equilibrated at 37°C for 30 minutes. The cannulated arteries were incubated with 2.5 μmol/L fura-2 AM in PSS at room temperature for 1 hour, followed by a washout period of 30 minutes at 37°C. Fura-2 fluorescence was measured by a photomultiplier system (IonOptix) with 2 alternated excitation wavelengths of 340 and 380 nm and an emission of 510 nm. The fluorescence intensity at each excitation wavelength (F₃₄₀ and F₃₈₀, respectively) and the ratio of these 2 fluorescence values (R₃₄₀/₃₈₀) were recorded simultaneously with arterial diameter measured with the use of the SoftEdge Acquisition Subsystem. Percent changes in [Ca²⁺]ᵢ, (measured as fura-2 R₃₄₀/₃₈₀) at each pressure step was determined as (Caᵢ - Caᵢ)/Caᵢ × 100, where Caᵢ was [Ca²⁺], obtained with normal PSS at given pressure and Caᵢ was [Ca²⁺], obtained with Ca²⁺-free PSS at the same pressure.

Data Analysis
Data are presented as means±SEM. Experimental number (n) represents offspring from different dams. The differences were evaluated for statistical significance (P<0.05) by 2-way ANOVA.

Results
Effect of Cocaine on Coronary Arterial Distensibility
There were no significant differences in the resting coronary arterial diameters between the control male (135.8±8.7 μm) and female (138.7±5.7 μm) offspring. Cocaine treatment had no significant effect on the resting diameters in either males (147.1±5.3 μm) or females (136.5±9.4 μm). As shown in

![Figure 1. Effect of cocaine on passive arterial diameters of coronary arteries. Pressure-induced passive arterial diameters were measured in Ca²⁺-free PSS in coronary arteries from 3-month-old male and female offspring that had been exposed in utero to saline control or cocaine. Data are means±SEM. *P<0.05, cocaine vs control. n=6 to 8 rats.](http://hyper.ahajournals.org/content/1124/6/1507/f1.large.jpg)

Figure 1, in the absence of extracellular Ca²⁺, arterial passive diameters significantly increased in response to step increases of intraluminal pressure (0 to 100 mm Hg). The pressure-mediated passive diameters were significantly greater in males than in females (P<0.05). Cocaine had no effect on the passive diameters in females but significantly decreased them in males (P<0.05; Figure 1). In the cocaine-treated animals, there were no significant differences in the passive diameters between male and female offspring.

**Effect of Cocaine on KCl-Induced [Ca²⁺]ᵢ and Contractions in Pressurized Coronary Arteries**
At intraluminal pressure of 20 mm Hg, addition of 120 mmol/L K-PSS caused a significant decrease in the vessel diameter, which was proceeded by an increase in [Ca²⁺]ᵢ (Figure 2A). Although there was no significant sex difference in KCl-mediated contractions, KCl-induced [Ca²⁺]ᵢ was significantly lower in female than in male arteries (Figure 2B and 2C). Cocaine had no significant effect on KCl-induced [Ca²⁺]ᵢ, and contractions in either males or females (Figure 2B and 2C).

**Effect of Cocaine on Pressure-Dependent Myogenic Response**
Coronary arteries of both male and female offspring developed myogenic tone in response to step increases of intralu-
minal pressure in the presence of extracellular Ca\(^{2+}\). In control animals, there was no significant difference in pressure-dependent myogenic tone between males and females in the absence of NG-nitro-L-arginine (L-NNA; Figures 3 and 4). In addition, L-NNA had no significant effect on the myogenic response in either control males (Figure 3) or females (Figure 4). In male offspring, prenatal cocaine caused a significant decrease in pressure-dependent myogenic tone in the absence or presence of L-NNA (Figure 3). In females, cocaine resulted in a significant increase in the myogenic tone of coronary arteries in the absence of L-NNA (Figure 4). In the presence of L-NNA, the myogenic tone was not significantly different between control and cocaine-treated female offspring (Figure 4). In cocaine-treated animals, pressure-dependent myogenic tone was significantly greater in female than in male offspring (Figures 3 and 4).

**Figure 2.** Effect of cocaine on KCl-induced constrictions and \([\text{Ca}^{2+}]_{i}\) in pressurized coronary arteries. KCl-induced changes in arterial diameters and \([\text{Ca}^{2+}]_{i}\) were measured simultaneously at intraluminal pressure of 20 mm Hg in coronary arteries from 3-month-old male and female offspring that had been exposed in utero to saline control or cocaine. A, Typical recordings of KCl-induced increase of \([\text{Ca}^{2+}]_{i}\), and decrease of the arterial diameter in the same tissue. B, KCl-induced increases of \([\text{Ca}^{2+}]_{i}\) (\([\Delta \text{Ca}^{2+}]_{i}\) R\(_{340/380}\)). C, KCl-induced decreases of the arterial diameter (\(\Delta\) diameter, \(\mu\)m). Data are means±SEM. *\(P<0.05\), female vs male. n=5 to 7 rats.

**Figure 3.** Effect of cocaine on myogenic tone in male coronary arteries. Pressure-dependent myogenic tone was determined in the absence or presence of L-NNA (100 \(\mu\)mol/L, 20 minutes) in coronary arteries from 3-month-old male offspring that had been exposed in utero to saline control or cocaine. Data are means±SEM. *\(P<0.05\), cocaine vs control. n=6 to 8 rats.

**Figure 4.** Effect of cocaine on myogenic tone in female coronary arteries. Pressure-dependent myogenic tone was determined in the absence or presence of L-NNA (100 \(\mu\)mol/L, 20 minutes) in coronary arteries from 3-month-old female offspring that had been exposed in utero to saline control or cocaine. Data are means±SEM. *\(P<0.05\), cocaine vs control. n=6 to 8 rats.
Effect of Cocaine on \([\text{Ca}^{2+}]_i\) in Pressurized Coronary Arteries

In the absence of extracellular \([\text{Ca}^{2+}]_i\), increasing pressure (10 to 100 mm Hg) did not affect \([\text{Ca}^{2+}]_i\) in coronary arteries of either male or female offspring (data not shown). In the presence of extracellular \([\text{Ca}^{2+}]_i\), step increases of intraluminal pressure significantly increased \([\text{Ca}^{2+}]_i\) in both males and females (Figure 5). Prenatal cocaine treatment had no significant effects on \([\text{Ca}^{2+}]_i\) of coronary arteries in either male or female offspring (Figure 5). To determine the effect of cocaine on the \([\text{Ca}^{2+}]_i\) sensitivity in the regulation of myogenic response of coronary arteries, the ratio of myogenic tone to \([\text{Ca}^{2+}]_i\), measured simultaneously in the same tissue, in response to step increase of intraluminal pressure was determined. As shown in Figure 6, prenatal cocaine significantly decreased the ratio of myogenic tone to \([\text{Ca}^{2+}]_i\) in male offspring but increased it in female offspring. In control animals, there was no significant difference in the ratio of myogenic tone to \([\text{Ca}^{2+}]_i\) between males and females, but it became significantly greater in female than in male offspring in cocaine-treated animals (Figure 6).

Discussion

The present study has demonstrated that prenatal cocaine exposure results in sex-dependent impairments in coronary myogenic response in adult offspring. Many previous studies have shown that adverse prenatal stimuli result in dysfunction of systemic vessels and an increased risk of hypertension in adult offspring. Nonetheless, only few studies examined fetal programming of vascular myogenic reactivity in offspring, despite its critical role in development of vascular tone and regulation of peripheral vascular resistance. It has been shown that fetal hypoxia causes an increase in pressure-dependent myogenic contractions of mesenteric arteries in male offspring. In addition, maternal undernutrition resulted in an increased myogenic response in the uterine artery. To our knowledge, the present study is the first to demonstrate in a rat model that adverse intrauterine environment causes an epigenetic modification of coronary vascular myogenic response in adult offspring. This finding is consistent with epidemiological studies showing an association of adverse intrauterine environment and an increased risk of coronary heart disease in adult life.

The finding of increased pressure-mediated passive diameters in male, as compared with female, vessels suggests a greater distensibility in male coronary arteries. Although this sex-difference has not been previously examined in small coronary arteries, a study in rats showed reduced stiffness of small cerebral arteries in males compared with females.
physiological significance of the finding remains to be elucidated, but the less distensibility of female coronary arteries may suggest a lower coronary flow reserve in females.\textsuperscript{14,15} Cocaine decreased pressure-mediated passive diameters in males and abolished the sex difference in coronary distensibility. In agreement, it has been shown that cocaine reduces coronary flow reserve in male dogs.\textsuperscript{16}

The lack of effect of cocaine on KCl-induced contractions of pressurized small coronary arteries in either females or males suggests that electromechanically-mediated contractile signal pathway in coronary arteries is not altered by fetal cocaine exposure. This is in agreement with the previous finding that prenatal cocaine had no effect on KCl-induced contractions of both aortas and resistance-sized mesenteric arteries in adult offspring.\textsuperscript{3} Similarly, another study showed that glucocorticoid exposure during early gestation had no significant effect on KCl-induced contractions in newborn lamb coronary arteries.\textsuperscript{17} In contrast, KCl-induced vascular contractility was enhanced in adult rats that exposed to nicotine before birth.\textsuperscript{18} These findings suggest a stimuli-specificity in fetal programming of electromechanic coupling in vascular smooth muscle in adult offspring.

The striking finding of opposite effects of fetal cocaine exposure on pressure-dependent myogenic response in male and female offspring is intriguing and suggests sex-specific fetal programming of coronary myogenic reactivity. The gender dichotomy in fetal programming of adult disease has been demonstrated in several animal models. Although the results are conflicting, it has been shown that female offspring are generally less sensitive in manifestation of cardiovascular disease caused by adverse prenatal stimuli.\textsuperscript{9} In the present study, we found that prenatal cocaine caused a significant decrease in coronary myogenic tone in male but an increase in female offspring. Pressure-induced myogenic tone is a major mechanism in coronary autoregulation.\textsuperscript{19–21} The heart is dependent on a continuous oxygen supply from the coronary circulation, as the myocardium has a very limited anaerobic capacity. Increased myocardial oxygen consumption in tachycardia and exercise is met principally by augmenting coronary blood flow. The striking decrease in coronary myogenic tone in cocaine-treated male offspring would predict a hemodynamic profile of “supranormal” coronary flow at rest and reduced coronary flow reserve, leading to an increased risk of ischemic heart disease when myocardial oxygen consumption increases several-fold in the transition from rest to exercise. In addition, a much dilated coronary circulation resulted from the decreased myogenic tone may cause abnormally high perfusion pressures to nutritive vessels and distort the balance of intravascular oncotic and hydrostatic pressure, increasing the risk of edema, ventricular stiffness, and poor ventricular performance. This notion is supported by the findings in cardiac allografts in human and animal studies showing that the decreased coronary myogenic tone led to a reduced coronary flow reserve and myocardial edema and stiffness.\textsuperscript{6,22}

In contrast to the finding in males, fetal cocaine exposure resulted in a significant increase in coronary myogenic tone in female offspring. Although the tightened myogenic regulation in females may suggest a greater capacity for coronary flow autoregulation and more effective buffering of capillary pressure from elevations in systemic pressure, it also impedes sufficient myocardial perfusion when oxygen demand increases. Indeed, the increased myogenic tone of coronary arteries is thought to play an important role in coronary heart disease in postmenopausal females.\textsuperscript{23}

Myogenic tone is an intrinsic property of smooth muscle and occurs independent of neural, metabolic, and endothelial influences. The role of nitric oxide (NO) in the regulation of vascular tone in the coronary resistance vessels is inconsistent, and numerous studies have shown that inhibition of NO synthesis with arginine analogs results in little or no change in coronary blood flow at rest or when myocardial oxygen consumption is elevated.\textsuperscript{24} In the present study, we found no effect of L-NNA on coronary myogenic tone in either male or female rats. The finding that cocaine-mediated reduction of myogenic tone was not affected by L-NNA in male offspring suggests a minimal role of NO in programming of coronary myogenic reactivity caused by cocaine in males. In contrast, in cocaine-treated female offspring, the increased coronary myogenic tone was blocked by L-NNA. This finding is surprising and suggests a stimulatory role of eNOS in the regulation of coronary myogenic tone in the cocaine-treated females. One possible explanation is that fetal cocaine exposure causes reprogramming of eNOS “uncoupling” in coronary arteries, thereby generating reactive oxygen species (ROS) such as superoxide rather than NO, resulting in the increased myogenic tone. This notion is supported by the recent findings that cocaine increased NADPH oxidase and ROS production in the heart.\textsuperscript{25–27} and that sustained elevation of NADPH oxidase and ROS led to eNOS uncoupling and endothelial dysfunction.\textsuperscript{28–30}

In the present study, we have demonstrated that cocaine-induced alterations in coronary myogenic tone are caused by changes in the Ca\textsuperscript{2+} sensitivity in both male and female offspring. This finding is consistent with growing evidence suggesting that mechanisms that regulate the Ca\textsuperscript{2+} sensitivity of the contractile apparatus in vascular smooth muscle form the backbone of pressure-induced myogenic vasoconstriction.\textsuperscript{31} Whereas the increased Ca\textsuperscript{2+} sensitivity in female offspring may be caused at least in part by aforementioned eNOS uncoupling and generation of ROS,\textsuperscript{31} distinct mechanisms are likely to be involved in the downregulation of the Ca\textsuperscript{2+} sensitivity in male offspring. We have recently demonstrated that prenatal cocaine results in protein kinase C ε (PKCe) gene repression via promoter methylation in the heart of male, but not female, offspring.\textsuperscript{32} Given that PKC plays an important role in the regulation of the Ca\textsuperscript{2+} sensitivity and myogenic tone,\textsuperscript{11} it is plausible to suggest that the downregulated PKC contributes to the decreased Ca\textsuperscript{2+} sensitivity in male coronary vessels.

**Perspectives**

The present finding of the long-term detrimental effect of prenatal cocaine exposure on coronary myogenic reactivity in adult offspring is in agreement with the recent findings that fetal cocaine results in the increased contractility of resistance vessels\textsuperscript{3} and heightened susceptibility of the heart to ischemia and reperfusion injury,\textsuperscript{2} and is consistent with other studies in
humans and animal models, demonstrating that multiple in utero adverse factors during gestation cause fetal programming and increase the risk of hypertension and coronary heart disease in the adult. Given that cocaine abuse in pregnant women is a significant problem, these findings should have a clinical significance and suggest that intrauterine cocaine exposure is a risk factor for morbidity and mortality secondary to myocardial ischemia in later life in offspring. The striking sex difference in programming of coronary myogenic reactivity warrants further investigation of possible roles of sex steroid hormones in fetal origins of coronary heart disease.

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Disclosures
None.

References
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