Fetal and Neonatal Nicotine Exposure Differentially Regulates Vascular Contractility in Adult Male and Female Offspring

DaLiao Xiao, Xiaohui Huang, Jennifer Lawrence, Shumei Yang, and Lubo Zhang

Center for Perinatal Biology, Department of Physiology and Pharmacology, Loma Linda University School of Medicine, Loma Linda, California (D.X., X.H., J.L., L.Z.); and Department of Chemistry and Biochemistry, California State University, San Bernardino, California (S.Y.)

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ABSTRACT

Epidemiologic studies suggest that prenatal exposure to maternal cigarette smoking is associated with an increased risk of elevated blood pressure in postnatal life. The present study was designed to test the hypothesis that fetal and neonatal nicotine exposure increased vascular contractility in adult offspring. Nicotine was administered to pregnant rats via s.c. osmotic minipumps throughout gestation and up to 10 days after delivery. Aortas were isolated from adult male and female offspring at the age of 3 months old. Nicotine significantly increased KCl- and norepinephrine-induced contractions of the aorta in male, but not female, offspring. Inhibition of endothelial nitric oxide synthase (eNOS) with N\^\text{G}-nitro-L-arginine (L-NNA) significantly increased norepinephrine-induced contractions in control male offspring but showed no effect in nicotine-treated male offspring. In the presence of L-NNA, there was no significant difference in norepinephrine-induced contractions between control and nicotine-treated males. In contrast, nicotine caused a significant increase in L-NNA-mediated potentiation of norepinephrine-induced contractions in female offspring. Nicotine had no effect on sodium nitroprusside-induced endothelium-independent relaxations of aortas from either male or female offspring. However, it decreased endothelium-dependent relaxations induced by acetylcholine in male offspring but increased them in females. There were no differences in eNOS protein levels in aortas between the control and nicotine-treated animals in either male or female offspring. The results suggest that fetal and neonatal nicotine exposure alters vascular functions in adult offspring in a gender-specific manner, which may lead to an increased risk of cardiovascular dysfunction in later life.

Nicotine is a major component of cigarette smoking. Adverse effects of nicotine on the cardiovascular system are well documented (Rejali et al., 2005). Nicotine can contribute to the development of cardiovascular disorders through a variety of mechanisms. It has been shown that the most significant effects of nicotine are through its influence on the vascular tone (Mayhan and Sharpe, 1996; Wang and Wang, 2000), the hemostatic system (Powell, 1998), and the endothelium (Mayhan et al., 1999). The combination of these effects leads to the development of various cardiovascular disorders such as atherosclerosis, cardiac arrhythmias, coronary artery disease, and hypertension. In addition, nicotine can cause alterations in the cellular growth, activities of regulatory proteins, and gene expression pattern of blood vessels (Sener et al., 2004). Although many nicotine-induced pathophysiological changes in the cardiovascular system have been determined, the specific mechanisms through which these pathologies occur have not been completely elucidated. In some cases, the effects of nicotine on a particular component of the cardiovascular physiology are controversial (Li et al., 1994; Clouse et al., 2000).

Maternal cigarette smoking is the single most widespread prenatal insult in the world. In the United States, one-fifth of pregnant women smoke. Smoking has long been associated with adverse pregnancy outcomes for the mother, her fetus, and newborn. The consequences have been well identified in epidemiological studies, including intrauterine growth retardation, sudden infant death syndrome, and persistent deficits in behavior of the offspring (Bulter and Goldstein, 1973; Naeye, 1992; Slotkin, 1998). Recent epidemiological studies have demonstrated that in utero exposure to maternal smoking is associated with elevated blood pressure and/or cardiovascular diseases in the offspring later in life (Beratis et al., 1996; Blake et al., 2000). However, it is difficult to infer a specific mechanism from epidemiology, and it is not yet clear...
whether nicotine itself is the major factor in these problems. Given that nicotine’s widespread use in tobacco products and in over-the-counter nicotine patches and gum, it is important to investigate effects of prenatal nicotine exposure on the cardiovascular system in postnatal life. It has been suggested that fetuses adapt to adverse intrauterine environmental influences by adjusting their physiological systems, i.e., altering the structure and function of specific tissues in the body (Barker and Martyn, 1992). These changes are likely beneficial/protective in the short term, but they may be maladaptive in later life. To understand the cardiovascular adaptation to prenatal nicotine exposure, the present study was designed to test the hypothesis that maternal nicotine administration during pregnancy increased vascular contractility and decreased endothelium-dependent vasorelaxation in adult offspring, which may lead to an increased risk of hypertension or other cardiovascular diseases in later life. The specific aims of the present study were to determine whether and to what extent prenatal and neonatal nicotine exposure affects KCl- and norepinephrine-induced contractions, endothelium-dependent and -independent vasorelaxations, and eNOS protein levels in the aorta of adult offspring. The studies were performed in both male and female offspring to investigate the potential gender effects of prenatal nicotine exposure.

Materials and Methods

Experimental Animals. Time-dated pregnant Sprague-Dawley rats were purchased from Charles River Laboratories (Portage, MI). Nicotine was administered through osmotic minipumps implanted s.c. as described previously (Slotkin, 1998; Bamford and Carroll, 1999). In brief, on the 4th day of pregnancy, rats were lightly anesthetized with ketamine and xylazine, and an incision was made on the back to insert osmotic minipumps (type 2ML4). The incision was closed with four sutures. Half of the pregnant rats were implanted with minipumps containing nicotine at a concentration of 102 mg/ml, and the other half were implanted with minipumps containing only saline, which served as the vehicle control. The flow rate of minipumps was 60 μl/day, which was delivered at a dose of 2.1 mg of nicotine free-base per day. In rats of an average of 350 g body weight, this corresponds to a dose rate of 6 mg/kg/day, which closely resembles those occurring in moderate to heavy human smokers (Lichtensteiger et al., 1988; Slotkin, 1999). According to the manufacturer’s specifications, the delivery period for the pumps is 28 days, so delivery continued after birth until postnatal day 10. Nicotine treatment did not affect the length of gestation, and all of the pregnancies reached their full term. Pups born to the dams were kept with their mothers until weaning. At that time, male and female pups were separated and transferred to cages where they were housed in groups of two. Male and female offspring were sacrificed at 3 months of age, and aortas were isolated for functional studies. All procedures and protocols used in the present study were approved by the Institutional Animal Care and Use Committee of Loma Linda University and followed the guidelines by the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Contraction Studies. The aortic segments were cut into 4-mm rings and mounted in 10-ml tissue baths containing a modified Krebs’ solution, pH 7.4, of the following composition: 115.2 mM NaCl, 4.7 mM KCl, 1.8 mM CaCl₂, 1.16 mM MgSO₄, 1.18 mM KH₂PO₄, 22.14 mM NaHCO₃, 0.03 mM EDTA, and 7.88 mM dextrose. The Krebs’ solution was oxygenated with a mixture of 95% O₂-5% CO₂. Isometric tension was measured in the Krebs’ solution in tissue baths at 37°C, as described previously (Xiao et al., 2001). After 60 min of equilibration, each ring was stretched to the optimal resting tension as determined by the tension developed in response to 120 mM KCl added at each stretch level. Norepinephrine-induced concentration-dependent contraction curves were obtained by cumulative addition of the agonist in approximate one-half log increments. In certain experiments, tissues were pretreated with a nitric oxide synthase inhibitor, Nω-nitro-l-arginine (t-NA; 100 μM, 20 min), as described in our previous study (Xiao et al., 2001b), and then stimulated with increased concentrations of norepinephrine. For relaxation studies, the tissues were precontracted with submaximal concentration (1 μM) of norepinephrine, followed by acetylcholine and sodium nitroprusside (SNP), respectively, added in a cumulative manner. The concentrations of norepinephrine, acetylcholine, and SNP were chosen to produce full concentration-response curves in the arteries.

Immunoblotting. eNOS protein levels were determined with Western blot analysis, as described previously (Xiao et al., 2001a). Tissues were homogenized in a lysis buffer containing 150 mM NaCl, 50 mM Tris.HCl, 10 mM EDTA, 0.1% Tween 20, 0.1% β-mercaptoethanol, 0.1 mM phenylmethylsulfonyl fluoride, 5 μg/ml leupeptin, and 5 μg/ml aprotinin, pH 7.4. Homogenates were then centrifuged at 4°C for 10 min at 10,000g, and the supernatants were collected. Protein was quantified in the supernatant using a protein assay kit from Bio-Rad ( Hercules, CA). Samples with equal protein were loaded on 7.5% polyacrylamide gel with 0.1% sodium dodecyl sulfate and separated by electrophoresis at 100 V for 2 h. Proteins were then transferred onto nitrocellulose membranes. Nonspecific binding sites in the membranes were blocked with overnight incubation at 4°C in a Tris-buffered saline solution containing 5% dry milk. The membranes were incubated with mouse eNOS monoclonal antibody, followed by secondary horseradish peroxidase-conjugated goat anti-mouse antibody. Proteins were visualized with enhanced chemiluminescence reagents, and the blots were exposed to Hyperfilm. Results were quantified with the Kodak electrophoresis documentation and analysis system and Kodak ID image analysis software (Eastman Kodak, Rochester, NY).

Immunohistochemistry. The aortic rings were fixed in 10% neutral buffered formalin and embedded in paraffin. Immunohistochemical detection of eNOS was performed using the Anti-Ig HRP Detection Kit (BD Biosciences PharMingen, San Diego, CA) as described previously (Kougias et al., 2006). Briefly, tissue slices (4 μm thick) of aortic rings were incubated with monoclonal anti-eNOS primary antibody (1:100) for 60 min at room temperature. After rinsing the slices three times in phosphate-buffered saline for 30 min, the slices were incubated with biotinylated goat anti-mouse IgG (1:100) for 60 min at room temperature. The samples were then exposed to streptavidin-HRP and reacted with diaminobenzidine substrate solution according to the manufacturer’s recommendations and counterstained with hematoxylin. The negative control of eNOS staining was performed in the absence of the eNOS antibody. The slices were viewed with an Olympus BH-2 microscope (Olympus, Tokyo, Japan), and images were captured with an attached SPOT digital camera imaging system.

Materials. Norepinephrine, l-NNa, SNP, nicotine hydrog tartrate, acetylcholine, and other chemicals were obtained from Sigma (St. Louis, MO). Osmotic minipumps (type 2ML4) were from Alza Corp. (Palo Alto, CA). Anti-Ig HRP Detection Kits were from BD Biosciences PharMingen. Mouse eNOS monoclonal antibody was from Transduction Laboratory (Lexington, KY). Electrophoresis and immunoblotting reagents were from Bio-Rad.

Data Analysis. Concentration-response curves were analyzed by computer-assisted nonlinear regression to fit the data using Prism (GraphPad Software, San Diego, CA) to obtain the values of pD₂ (-log EC₅₀) and the maximal response. Results were expressed as means ± S.E.M., and the differences were evaluated for statistical significance (P < 0.05) by two-way analysis of variance followed by Bonferroni’s post-tests.
Results

Effect of Nicotine on Litter Size and Body Weight. Fetal and neonatal nicotine treatment showed no effect on the litter size at birth (control, 13.2 ± 0.4, n = 9; nicotine, 13.6 ± 0.4, n = 8; P > 0.05). However, the birth weights of animals from nicotine-treated mothers (5.8 ± 0.1 g, n = 4) were significantly reduced as compared with those from control mothers (6.9 ± 0.1, n = 4; P < 0.05). As shown in Fig. 1, there were no significant differences between the control and nicotine treatment in body weight of the male and female adult offspring at the age of 3 months old.

Effect of Nicotine on KCl- and Norepinephrine-Induced Contractions in Adult Offspring. Figure 2 shows the effect of fetal and neonatal nicotine exposure on KCl-induced contractions of aortas in adult male and female offspring at the age of 3 months old. As shown in Fig. 2, KCl-induced contractions were significantly increased in nicotine, as compared with control groups in the male (nicotine, 2.7 ± 0.2 versus control, 1.6 ± 0.1 g/mm²; P < 0.05) but not the female (nicotine, 2.2 ± 0.2 versus control, 2.1 ± 0.2 g/mm²; P > 0.05), offspring. There was no significant difference in KCl-induced contractions between control male and female animals.

Figure 3 shows the effect of nicotine exposure on norepinephrine-induced concentration-dependent contractions of aortas in the absence or presence of the eNOS inhibitor l-NNA in adult male offspring. In the absence of l-NNA, the pD₂ values of norepinephrine-induced contractions were not significantly different between the control and nicotine-treated animals, but the maximal response was significantly increased in the aorta of nicotine-treated animals, as compared with that of the control (Table 1). In the control animals, inhibition of eNOS with l-NNA significantly potentiated norepinephrine-induced contractions and increased the norepinephrine-mediated maximal response (Table 1). In contrast, in nicotine-treated animals, norepinephrine-induced contractions were not significantly affected by l-NNA.

In the presence of l-NNA, there was no significant difference in norepinephrine-induced contractions between the control and nicotine-treated animals (Table 1). In contrast to male offspring, fetal and neonatal nicotine exposure showed no effect on norepinephrine-induced contractions of the aorta in adult female offspring (Fig. 4; Table 2). In the control animals, inhibition of eNOS with l-NNA had no significant effect on the pD₂ values of norepinephrine-induced contractions but slightly increased the maximal response (Fig. 4). On the other hand, in nicotine-treated animals, l-NNA caused a nonparallel leftward shift of the norepinephrine dose-response curve and significantly increased the contractile potency of norepinephrine by nearly 50-fold (Fig. 4; Table 2). In the presence of l-NNA, there was a 22-fold increase in the potency of norepinephrine-induced contractions of the aorta in the nicotine-treated female off-

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Fig. 1. Effect of prenatal nicotine on body weight of adult offspring. Bars graphs, body weight of male and female adult offspring that had been exposed to saline control or nicotine before birth. Data are means ± S.E.M. of from 10 to 12 animals in each group.

Fig. 2. Effect of prenatal nicotine on KCl-induced contractions of aortas from adult offspring. KCl (120 mM)-induced contractions were measured in aortas from male and female adult offspring that had been exposed to saline control or nicotine before birth. Data are expressed as active tension (grams) per cross-section area (millimeters squared) of aortic rings. Data are means ± S.E.M. of tissues from five to eight animals in each group. *, P < 0.05, nicotine versus control.

Fig. 3. Effect of prenatal nicotine on norepinephrine-induced contractions of aortas from adult male offspring in the absence or presence of l-NNA. Norepinephrine-induced contractions were measured, in the absence or presence of l-NNA (100 μM, 20 min), in aortas from male adult offspring that had been exposed to saline control or nicotine before birth. Data are expressed as percent KCl (120 mM)-induced contractions and are means ± S.E.M. of tissues from seven to eight animals in each group. The pD₂ values and the maximal response are presented in Table 1.

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Xiao et al.
spring, as compared with that of the control animals (Table 2).

**Effect of Nicotine on Endothelium-Dependent Relaxation in Adult Offspring.** The endothelium-dependent relaxation induced by acetylcholine was examined in aortas precontracted with 1 μM norepinephrine. In male offspring, acetylcholine produced concentration-dependent relaxations in both control and nicotine-treated animals (Fig. 5, top). The maximal relaxation induced by acetylcholine was significantly decreased in nicotine-treated animals, as compared with that in the control (Table 3). Unlike acetylcholine, sodium nitroprusside-induced, endothelium-independent relaxations were not significantly different between the control and nicotine-treated animals (Fig. 5, bottom).

In contrast to male offspring, the maximal relaxation induced by acetylcholine was significantly increased in female adult offspring that exposed to nicotine in fetal and neonatal period, as compared with that in the control (Fig. 6, top; Table 3). Consistent with the finding in male offspring, fetal and neonatal nicotine exposure showed no significant effect on sodium nitroprusside-induced relaxations in female adult offspring (Fig. 6, bottom).

**Effect of Nicotine on eNOS Expression in Adult Offspring.** To determine whether eNOS expression was correlated with the alteration of endothelium-dependent relaxations in adult offspring caused by fetal and neonatal nicotine exposure, eNOS protein levels in aortas were determined by Western blotting. As shown in Fig. 7, eNOS immunoreactivity was exclusively detected in the endothelium of the aorta. eNOS protein levels in aortas were not significantly different between the control and nicotine-treated animals of either male or female offspring (Fig. 8).

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**TABLE 1**

Effect of pre- and postnatal nicotine exposure on norepinephrine-mediated contractions of aorta in male adult offspring in the absence or presence of l-NNA. 

<table>
<thead>
<tr>
<th>Control</th>
<th>Nicotine</th>
</tr>
</thead>
<tbody>
<tr>
<td>pD₂</td>
<td>Eₘₐₓ</td>
</tr>
<tr>
<td>l-NNA</td>
<td>7.53 ± 0.16</td>
</tr>
<tr>
<td>+l-NNA</td>
<td>7.71 ± 0.11</td>
</tr>
</tbody>
</table>

*P < 0.05, +l-NNA vs. −l-NNA.

**TABLE 2**

Effect of pre- and postnatal nicotine exposure on norepinephrine-mediated contractions of aorta in female adult offspring in the absence or presence of l-NNA. 

<table>
<thead>
<tr>
<th>Control</th>
<th>Nicotine</th>
</tr>
</thead>
<tbody>
<tr>
<td>pD₂</td>
<td>Eₘₐₓ</td>
</tr>
<tr>
<td>l-NNA</td>
<td>7.76 ± 0.12</td>
</tr>
<tr>
<td>+l-NNA</td>
<td>8.07 ± 0.11</td>
</tr>
</tbody>
</table>

*P < 0.05, +l-NNA vs. −l-NNA.

**Fig. 4.** Effect of prenatal nicotine on norepinephrine-induced contractions of aortas from adult female offspring in the absence or presence of l-NNA. Norepinephrine-induced contractions were measured, in the absence or presence of l-NNA (100 μM, 20 min), in aortas from female adult offspring that had been exposed to saline control or nicotine before birth. Data are expressed as percent KCl (120 mM)-induced contractions and are means ± S.E.M. of tissues from eight animals in each group. The pD₂ values and the maximal response are presented in Table 2.

**Fig. 5.** Effect of prenatal nicotine on acetylcholine (Ach)- and SNP-induced relaxations in aortas from adult male offspring. Ach- and SNP-induced relaxations were measured in aortas from male adult offspring that had been exposed to saline control or nicotine before birth. Aortic rings were precontracted with 1 μM norepinephrine, followed by a cumulative addition of Ach or SNP. Data are means ± S.E.M. of tissues from seven to eight animals in each group. The pD₂ values and the maximal response are presented in Table 3.
The major findings of the present study are prenatal and neonatal nicotine exposure increased both KCl- and norepinephrine-induced contractions of aortas in male, but not female, adult offspring; inhibition of eNOS by L-NNA increased norepinephrine-induced contractions in both male and female control rats, however, the effect of L-NNA was abolished in male but enhanced in female offspring of nicotine-treated animals; the nicotine treatment decreased the endothelium-dependent relaxation in male offspring but increased it in female offspring; unlike the endothelium-dependent relaxation, the endothelium-independent relaxation induced by SNP was not significantly affected by nicotine in either male or female offspring; and eNOS protein levels were not significantly different between control and nicotine-treated animals of male and female offspring. Cigarette smoking/nicotine has deleterious effects on the cardiovascular system (Toda et al., 1995; Lambers and Clark, 1996; Benowitz, 1997; Sener et al., 2004). To our knowledge, the present study is the first to show in a rat model that fetal and neonatal exposure to nicotine causes an epigenetic modification of vascular contractility in adult offspring. The finding of reduced birth weight in nicotine-treated animals is in agreement with previous studies (Bamford and Carroll, 1999; Gauda et al., 2001; Simakajornboon et al., 2004). Recent studies suggested that decreased birth weight from maternal nicotine exposure resulted from direct effects of nicotine rather than indirect effects due to placental insufficiency or fetal hypoxia (Huang et al., 2006). Nicotine crosses the placenta with fetal blood levels 15% greater than those of the mother (Lambers and Clark, 1996). In the present study, the maternal infusion of nicotine through osmotic minipumps occurred during most of the prenatal period and continued for approximately 10 days after birth. Although this raised the possibility that the pups continued to be exposed to nicotine through mothers’ milk, previous studies using the

Table 3

Effect of pre- and postnatal nicotine exposure on acetylcholine-induced endothelial dependent relaxation of aorta in male and female adult offspring.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Nicotine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>pD2</td>
<td>E_max</td>
</tr>
<tr>
<td></td>
<td>7.25 ± 0.10</td>
<td>70.4 ± 2.3</td>
</tr>
<tr>
<td>Female</td>
<td>7.11 ± 0.17</td>
<td>56.3 ± 2.9</td>
</tr>
</tbody>
</table>

<sup>a</sup> P < 0.05, male vs. female.

<sup>b</sup> P < 0.05, nicotine vs. control.

Discussion

The major findings of the present study are prenatal and neonatal nicotine exposure increased both KCl- and norepinephrine-induced contractions of aortas in male, but not female, adult offspring; inhibition of eNOS by L-NNa increased norepinephrine-induced contractions in both male and female control rats, however, the effect of L-NNa was abolished in male but enhanced in female offspring of nicotine-treated animals; the nicotine treatment decreased the endothelium-dependent relaxation in male offspring but increased it in female offspring; unlike the endothelium-dependent relaxation, the endothelium-independent relaxation induced by SNP was not significantly affected by nicotine in either male or female offspring; and eNOS protein levels were not significantly different between control and nicotine-treated animals of male and female offspring.
and in the presence of L-NNA, there was no significant difference in norepinephrine-mediated contractions between control and nicotine-treated animals. This finding indicates that the enhanced norepinephrine-induced contractions of aortas in male offspring of nicotine-treated animals are primarily due to the loss of the eNOS-mediated relaxation component, rather than increased norepinephrine-induced contractions per se, in adult vessels. The finding that the nicotine treatment attenuated acetylcholine-, but not nitroprusside-, induced relaxations in male offspring reinforces the notion that the observed alterations in the vascular reactivity are a consequence of primary changes in endothelium-mediated pathways. It has been well documented that adverse intrauterine environments may cause fetal programming, resulting in decreased endothelium-dependent vasodilatation in human and animal models (Franco et al., 2002, 2003; Lamireau et al., 2002; Brawley et al., 2003; Morman and Martin, 2003).

To further evaluate potential endothelium-dependent mechanisms underlying the observed alterations, eNOS protein expression and localization were examined in aortas. It is not a surprise that eNOS was detected only in the endothelium of aortic rings, demonstrating an endothelium location of eNOS. The finding of no significant difference of eNOS levels suggests that the decreased endothelium-dependent relaxation in male offspring of nicotine-treated animals results primarily from a decrease in eNOS activity. This is in agreement with the previous studies in rats showing that intrauterine malnutrition decreased eNOS activity and nitric oxide production without affecting eNOS gene expression (Franco et al., 2004). The eNOS activity is regulated by several factors, including caveolin-1, heat shock protein 90, and bioavailability of tetrahydrobiopterin (BH4). Although there is no evidence showing that nicotine affects caveolin-1 or heat shock protein 90, it has been shown that smoking causes dysfunctional eNOS due to a reduced bioactivity of BH4 (Heitzer et al., 2000; Ueda et al., 2000). In addition, it has been demonstrated that the impairment of BH4 played an important role in decreased eNOS activity in offspring of intrauterine undernourished rats (Franco et al., 2004). Taken together, these findings suggest a potential role of BH4 in fetal programming of endothelial eNOS activity in response to nicotine exposure.

In contrast to male offspring, neither KCl- nor norepinephrine-induced contractions were affected in female offspring of nicotine-treated animals. The gender dichotomy in manifestation of the severity of hypertension in adult offspring has been observed in animal models of intrauterine undernutrition, with the male being more susceptible than the female (Franco et al., 2003). A few studies examined gender differences in vascular reactivity in rats and showed that impairment of endothelium-dependent relaxation was more pronounced in male than female offspring that experienced intrauterine undernutrition (Ozaki et al., 2001; Franco et al., 2002). In the present study, we have found that in control female offspring, inhibition of eNOS with L-NNA slightly increased the maximal response of norepinephrine-induced contractions, with no effect on the pD<sub>2</sub> value. To our surprise, in nicotine-treated animals, L-NNA caused a nonparallel leftward shift of the norepinephrine dose-response curve and significantly increased the contractile potency of norepinephrine by nearly 50-fold. Consistent with the finding in male
offspring, eNOS protein levels in aortas were not significantly different in female offspring between control and nicotine-treated animals. These findings suggest that prenatal nicotine exposure significantly increased eNOS activity in female offspring. This is further supported by the finding that acetylcholine-, but not nitroprusside-, induced relaxations of aortas were significantly increased in female offspring of nicotine-treated animals.

In addition to increased eNOS activity, fetal and neonatal nicotine exposure significantly increased nitric oxide-dependently increased contractions of aortas in female offspring. This is supported by the finding that in the presence of L-arginine, there was a 22-fold increase in the potency of norepinephrine-induced contractions of aortas in nicotine-treated animals. Although the normal vascular reactivity/tone may be maintained through the compensatory effect of increased eNOS activity in endothelium-intact vessels, the markedly enhanced nitric oxide-dependently increased contractions in female offspring of nicotine-treated animals likely significantly increase the vulnerability of vasospasm in pathophysiological conditions of endothelium/eNOS dysfunction in female offspring of nicotine-treated animals.

It is not clear at present whether acetylcholine receptors may contribute to the alterations of acetylcholine-induced relaxation of aortas in male and female offspring, observed in the present study. It has been shown that changes in nicotine receptors in the brain, caused by prenatal nicotine exposure, may play a role in sex differences in behavioral and neurochemical responses in offspring (Tizabi et al., 1997; Slotkin, 1998). To our knowledge, the effects of prenatal nicotine exposure on acetylcholine receptors in the vasculature of offspring have not been reported and present an area for further investigation.

In summary, we demonstrated that vascular responses in adult offspring were affected and programmed by fetal and neonatal nicotine exposure. In addition, we demonstrated a gender dichotomy in programming of vascular reactivity. In male offspring, the nicotine treatment caused a decreased endothelial eNOS activity but had no effect on nitric oxide-dependently increased contractions per se. In contrast, in female offspring, the nicotine treatment caused an increase in both endothelial eNOS activity and nitric oxide-dependently increased contractions. Given the findings that prenatal nicotine increased testosterone levels in female offspring (Smith et al., 2003) and that testosterone increased both α-adrenoceptor-mediated contractions and endothelial eNOS activity (Thiyagarajan et al., 2002; Bai et al., 2005; Martin et al., 2005), the potential roles of sex hormones in the nicotine-mediated programming of adult vascular reactivity warrant further study.

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increases testosterone levels in the fetus and female offspring. *Nicotine Tob Res* 5:369–374.


Address correspondence to: Dr. DaLiao Xiao, Center for Perinatal Biology, Department of Pharmacology and Physiology, Loma Linda University School of Medicine, Loma Linda, CA 92350. E-mail: dxiao@llu.edu