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Low doses of nicotine-induced fetal cardiovascular responses, hypoxia, and brain cellular activation in ovine fetuses

Junchang Guan [a,b,1], Caiping Mao [a,1], Feicao Xu [a,1], Liyan Zhu [a], Yujuan Liu [a], Chongsong Geng [a], Lubo Zhang [a,c], Zhice Xu [a,c,*]

[a] Perinatal Biology Center, Soochow University School of Medicine, Suzhou, China
[b] Department of Microbiology, Bengbu Medical College, Bengbu, Anhui, China
[c] Center for Prenatal Biology, Loma Linda University School of Medicine, CA 92350, USA

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ABSTRACT

Prenatal exposure to nicotine is associated with a variety of adverse outcomes. The present study investigated the effect of low doses of nicotine during pregnancy on fetal blood gases, cardiovascular system, and cellular activation in the brain. Intravenous administration of nicotine 10 or 25 μg/kg into ewe did not affect maternal blood gases, blood pressure, and heart rate. Maternal administration of nicotine also had no effect on fetal blood electrolyte concentrations, osmolality levels, and lactic acid levels. However, it significantly reduced fetal blood pO_2 levels and oxygen saturation, increased fetal arterial blood pressure and decreased heart rate *in utero*. In addition, exposure to low doses of nicotine increased the expression of Fos in the paraventricular nucleus (PVN) and subfornic organ (SFO) in the fetal brain. The data demonstrated that even low doses of nicotine could impact significantly on fetal cardiovascular and central nervous systems, as well as oxygen status, and suggested a toxic risk to fetuses of exposure to low levels nicotine or second-hand smoking during pregnancy.

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1. Introduction

Prenatal exposure to nicotine is closely associated with a variety of adverse outcomes, including premature delivery, low birth weight, increased perinatal mortality, and sudden infant death syndrome (Salihu et al., 2003; Pollack et al., 2000; Coleman et al., 2004). Several studies have shown the direct and indirect effects of maternal smoking on fetus *in utero*. Exposure to nicotine during pregnancy can induce fetal intrauterine hypoxia (Mao et al., 2008; Suzuki et al., 1971). In adults, nicotine can elevate arterial blood pressure and heart rate (Westman, 1995). Although it is well documented that larger doses of nicotine (like the situation of moderate to heavy smoking) can change fetal heart rate and umbilical blood flow (Lindblad et al., 1988; Kirschbaum et al., 1970), and induce hypoxia in the fetus (Mao et al., 2008; Suzuki et al., 1971), it is largely unknown of effects of low levels of nicotine on fetal cardiovascular system. Thus, we determined the effect of administration of low doses of nicotine in maternal sheep on fetal cardiovascular responses and *in utero* oxygen status in the present study.

In adults, nicotine as a toxic chemical can cause cellular damage or pathophysiological changes in the brain (Abreu-Villaca et al., 2003; Eisenblätter et al., 2003). Exposure to smoking or nicotine during pregnancy also could affect fetal central nervous system (CNS) and brain development (Lv et al., 2008). Although previous studies have shown that application of nicotine could affect fetal blood pressure or heart rate (Lindblad et al., 1988; Kirschbaum et al., 1970), it is unknown whether exposure to nicotine during pregnancy would influence the subfornical organ (SFO) and the paraventricular nuclei (PVN) in the fetal brain. Notably, those and other nuclei are critical in the brain in the control of cardiovascular systems (Fernandes et al., 2007). Addressing this question will provide new information not only for understanding influence of smoking or nicotine on the fetal brain, but also for further determination of mechanisms of nicotine-induced cardiovascular changes. Therefore, the experiments in the present study also were designed in determination the effect of exposure to low doses of nicotine on cellular activation in the fetal brain.

2. Materials and methods

Twenty time-dated pregnant ewes with singleton fetuses (128 ± 3 days of gestation on the study day) were used. Animals were housed in individual cages in a light-controlled room (12:12-h light–dark cycle), with food and water provided *ad libitum*.

* Corresponding author at: Perinatal Biology Center, Soochow University School of Medicine, Suzhou, China. Tel.: +86 512665880125.
E-mail address: xuzhice@suda.edu.cn (Z. Xu).
[1] Those authors and those Institutes (a.b) contributed equally to this work.

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2.1. Surgery

Anesthesia was induced with ketamine hydrochloride (20 mg/kg im), and general anesthesia was maintained with 1–2% isoflurane and 1 l/min oxygen. Polyethylene catheters were placed in the femoral artery and vein and threaded to the inferior vena cava and abdominal aorta, respectively, of both the fetus and the ewe, as previously reported (Xu et al., 2005). All catheters were externalized to the maternal flank and placed in a cloth pouch. Animals were allowed 4 days of postoperative recovery, which included catheter maintenance and antibiotic administration.

2.2. Experimental design

Animals were studied from 8:00 AM to 12:00 PM. Studies began with a baseline period (−60 to 0 min) followed by study period (0–120 min). Pregnant ewes were divided into four groups randomly. In the experimental animals, 10, 25, or 750 μg/kg of nicotine hydrogen tartrate (Sigma, St. Louis, MO) in 10 ml 0.9% NaCl was infused intravenously to the maternal ewe (1 ml/min) over 10 min, respectively. Doses of nicotine were referred to previous reports and our preliminary studies (Mao et al., 2008; Suzuki et al., 1971). Control group was infused intravenously with the same volume of saline (0.9% NaCl) at the same speed. All solutions were freshly prepared. After infusions, animals were continually monitored for additional 120 min.

Throughout the study, maternal and fetal blood pressures and heart rates were measured continuously. Maternal and fetal blood samples were collected at the baseline period and at 15, 30, 90, and 120 min after initiation of the infusion. Meanwhile, fetal urine sample was collected in every 30 min at the baseline period and after initiation of the infusion. Fetal and maternal blood pressures were measured by means of a Power-Lab multichannel recorder (AD Instruments, Australia). Maternal and fetal blood samples were collected into iced tubes containing lithium heparin. Blood samples were determined for pH, pO_2, pCO_2, oxygen saturation (SO_2%), hematocrit (Hct), hemoglobin (Hb) level, glucose (Glu), and lactic acid (Lac) levels, as well as Cl^-, sodium (Na^+) and potassium (K^+) concentrations with a Nova analyzer (Nova Biochemical, Model pHOx Plus L, Waltham, MA) at 39 °C base. The remaining blood was centrifuged at 4 °C for 15 min. Then plasma was used for measuring osmolality with an advanced digmatic osmometer (model 3MO, Advanced Instruments, Needlham Heights, MA). Fetal urine samples were collected into tube for measurement of urine volume, pH, osmolality, Na^+, K^+, Cl^-, and Ca^{++} concentrations.

2.3. Fos immunostaining

Two groups of animals (the control group: maternal infusion with 0.9% NaCl, and the experimental group: maternal infusion with 25 μg/kg of nicotine) were used. At the end of the study described above, the ewes were anesthetized with ketamine anesthesia (20 mg/kg iv) and ventilated with a mixture of isoflurane and oxygen. A middle abdominal incision was made, and the fetal head and neck were exposed. A 16-gauge needle was inserted into one side of the fetal carotid artery for perfusion. The fetuses were perfused via carotid artery with 0.1 M PBS followed by 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer. Postfixation was performed in PFA solution for 12–24 h, after which brains were placed in 20% sucrose in 0.01 M phosphate overnight. Thirty-micrometer coronal sections of the fetal brain were cut through the forebrain on a cryostat. Every other section of the brain was used for immunocytochemistry staining with the avidin-biotin peroxidase technique. The primary antibodies (Santa Cruz Biotech, Santa Cruz, CA) were raised from rabbits against Fos protein. The tissue sections were incubated for 1 h at room temperature on a gentle shaker and then overnight at 4 °C in the primary antibody (primary antibody with 0.3% Triton X-100). The sections were further incubated in goat anti-rabbit serum (1:500) and then processed using the Vectastain ABC kit (Vector Labs, "Elite" ABC reagent, Burlingame, CA). The sections were treated with 1 mg/ml diaminobenzidine tetrahydrochloride (Sigma) (0.02% hydrogen peroxide). All sections were on slides, dehydrated in alcohol, and then were coverslipped.

2.4. Data analysis

The number of c-Fos positive cells in fetal brain sections was evaluated in a qualitative manner by microscopy analysis, as reported before (Rinaman et al., 1997). The regions counted were the fetal paraventricular nucleus and subfornic organ in the forebrain. A repeated measures of ANOVA was used to determine differences over time and effects of treatments. Comparison of before and after treatments was determined with posthoc test. All data are expressed as means ± SE.

3. Results

3.1. Blood values and electrolytes

There was no significant difference in maternal blood pO_2, pCO_2 and SO_2% between the control and treated groups (Fig. 1A–C). However, following infusion of nicotine into the ewe, a significant change of the fetal blood pO_2, pCO_2, and SO_2% was observed between the control and treated groups. 10 or 25 μg/kg nicotine hydrogen tartrate significantly decreased fetal blood pO_2 and SO_2%, and increased fetal blood pCO_2 from 30 to 120 min following infusions (Fig. 2A–C). Both maternal and fetal blood values (including pH, Hb, Hct, Glu, and Lac) as well as electrolytes (including Na^+, K^+, Cl^- concentrations) and osmolality were not changed in response to the nicotine infusions (Tables 1–4). Notably, the infusion of 750 μg/kg nicotine hydrogen tartrate to ewes ($n = 2$) pronouncedly decreased fetal blood pO_2 from 20.8 to 11.5 mmHg, the fetuses were aborted on the following day after the nicotine infusion.

3.2. Urine electrolytes and osmolality

Between the control and treated groups, there was no significant difference in fetal urine values, including urine volume, pH, osmolality, Na^+, K^+, Cl^- and Ca^{++} concentrations (Table 5).

3.3. Cardiovascular response

Maternal arterial blood pressures [systolic (SBP) and diastolic pressure (DBP) and mean arterial pressure (MAP)] and heart rate (HR) did not change significantly in response to either 0.9% NaCl or nicotine infusions (Table 6). In response to the maternal infusion of nicotine, fetal SBP, DBP and MAP significantly increased from 30 min following the infusion (Fig. 3A–C). Although infusion of 10 μg/kg nicotine hydrogen tartrate did not significantly increase fetal HR, 25 μg/kg nicotine hydrogen tartrate significantly reduced fetal HR from 30 min after the infusion of nicotine (Fig. 3D).

3.4. Expression of c-Fos protein

In the control fetuses infused with isotonic saline, there were little positive Fos proteins in the fetal brain. However, maternal intravenous nicotine produced intense Fos in the fetal brain. The areas with positive Fos staining following nicotine infusion included the SFO, organum vasculosum of lumina terminalis (OVLT), median preoptic nucleus (MePO), and the supraoptic