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

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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 pO₂ 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.

Keywords

Blood pressure; c-Fos expression; Neurotoxin

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.

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Conflict of interest None.

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*.

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 $\mu\text{g/kg}$ 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 ($\text{SO}_2\%$), 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, Needham 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 pO₂, pCO₂ and SO₂% between the control and treated groups (Fig. 1A–C). However, following infusion of nicotine into the ewe, a significant change of the fetal blood pO₂, pCO₂, and SO₂% was observed between the control and treated groups. 10 or 25 µg/kg nicotine hydrogen tartrate significantly decreased fetal blood pO₂ and SO₂%, and increased fetal blood pCO₂ 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 pO₂ 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 nucleus (SON) and PVN. There were significant differences of positive Fos proteins in the fetal SFO and PVN between the isotonic- and nicotine-infused fetuses (Figs. 4 and 5).

4. Discussion

This study investigated the effect of maternal use of nicotine on the fetal oxygenic status, cardiovascular system, and cellular activation in the fetal brain. We found that intravenous infusion of low doses of nicotine in maternal sheep induced fetal hypoxia, elevated fetal blood pressure, and increased the expression of Fos protein in the fetal central nervous system. Notably, this was the first *in utero* study with direct measurements in demonstration of that exposure to low levels of nicotine could still cause fetal hypoxia in association of cardiovascular responses and brain cellular activation.

In the present study, two pregnant sheep were infused with the larger dose of nicotine (750 µg/kg) intravenously. However, their fetuses were aborted within 24 h following use of nicotine, indicating risk of exposure to nicotine for premature delivery and perinatal mortality. This was consistent with previous reports (Pollack et al., 2000; Nielsen et al., 2006). Nicotine can easily cross the placenta from the maternal circulation into the fetus and concentrate in fetal tissue (Ankarberg et al., 2001). Previous studies (Suzuki et al., 1974; Dempsey and Benowitz, 2001) showed that nicotine concentrations in mother and fetus were similar and persisted for a longer period of time in the fetus than that in the mother. In the present study, intravenous infusion of low doses of nicotine affecting fetal (not maternal) oxygen status and cardiovascular systems indicates the sensitivity and vulnerability of the fetus to nicotine (Pollack et al., 2000).

In animal fetuses, a decreased fetal blood pO₂ following nicotine infusions (higher doses) in the maternal compartment has been reported. Subcutaneous administration of nicotine 6 mg/kg/day to maternal rats from the early gestation increased fetal hemoglobin associated with reduction of pO₂ (Mao et al., 2008; Suzuki et al., 1971). Nicotine administrated intravenously (1 mg/kg) into the maternal rhesus monkeys also caused a significant drop in fetal pO₂ (Suzuki et al., 1971). In pregnant sheep (Manning et al., 1978), nicotine at the dose of 0.14–0.25 mg/kg injected in maternal carotid artery and jugular vein also lowered fetal oxygen levels. Notably, those studies used nicotine either in chronic manner or with large doses. In the present study, we demonstrated that the low doses of nicotine (10 and 25 µg/kg) also could impact significantly on fetal blood gases, indicating a higher risk to fetuses from exposure to nicotine, and suggesting a risk to fetuses from second-hand smoking during pregnancy (nicotine levels are usually lower in second-hand smoking than that in first hand smoking).

In the present study, the low doses of nicotine also induced a significant elevation of fetal blood pressure. Automatic pathways play an important role in cardiovascular regulation. In adults, nicotine elevated arterial blood pressure and heart rate (Najem et al., 2006; Westman, 1995). The mechanism for increase of blood pressure and heart rate by nicotine was linked to activation of the sympathetic nervous system with release of norepinephrine and epinephrine (Cryer et al., 1976). Cigarette smoking resulted in sympathetic neural arousal that lasted for 24 h (Benowitz et al., 1984). In the present study, maternal administration of nicotine significantly elevated fetal blood pressure. Although it needs further studies to clarify if peripheral sympathetic activation also plays a role in such cardiovascular responses in the fetus, the results from our Fos study indicated that the central nuclei in the control of autonomic pathways were activated by exposure to maternal nicotine, which may contribute to the increased fetal blood pressure.

The cardiovascular center in the brain regulates blood pressure via the autonomic pathways. *c-Fos*, an immediate early gene and its protein, has been extensively used as a marker for cellular and neural activation in the CNS (Sagar et al., 1988). A line of studies have shown that administration of nicotine can induce Fos expression in restricted brain cells and regions in rats, including midbrain dopamine neurons and in the hypothalamic areas (Seppä et al., 2001; Shram et al., 2007). The present study provided new information that exposure to maternal nicotine could induce intensive Fos expression in the fetal SFO and PVN *in utero*, while no or few Fos was present in the control brain. Those two nuclei have been demonstrated as important brain areas in the control of cardiovascular activity (Fernandes et al., 2007). It was possible that maternal administration of nicotine-induced brain activation contributed to an increase of fetal blood pressure at near-term via central pathways.

As mentioned above, nicotine can quickly enter fetal circulation from the maternal side (Ankarberg et al., 2001; Suzuki et al., 1974; Dempsey and Benowitz, 2001), and produce *in utero* hypoxia as observed in the present study. Several studies have suggested that hypoxia also can induce expression of Fos in the brain (Trauth et al., 1999; Ness et al., 2008). In addition, nicotine in the peripheral circulation can cross the blood-brain barrier (Eisenblätter et al., 2003) to act on the CNS directly. A question raised immediately was whether nicotine itself or nicotine-induced hypoxia caused cellular activation in the fetal brain in the present study. This opens new opportunities for nicotine studies in fetal neurophysiology and pathophysiology with sampling blood across the placenta to assess placental perfusion.

Hypoxia can cause an increase of blood pressure (Barton et al., 2001; Calbet, 2003; Fletcher et al., 2002). For example, one study (Barton et al., 2001) showed that hypertension induced by chronic hypoxia was associated with down-regulation of nitric oxide synthase. Chronic hypoxia also increased systemic arterial pressure in healthy humans (Calbet, 2003). Taken together, several routes and possibilities could be counted in fetal cardiovascular changes following exposure to nicotine during pregnancy. First, nicotine-mediated central activation in the cardiovascular center may contribute importantly to the fetal pressor response since the fetal SFO and PVN were activated; Second, nicotine-induced hypoxia may increase fetal blood pressure *in utero*; Third, nicotine itself may act directly in the peripheral systems in stimulating cardiovascular systems.

It is well known that other factors also may be involved in cardiovascular changes following exposure to nicotine. For example, nicotine may modulate cholinergic signals in affecting body fluid regulatory systems that could influence vascular volume and pressure (Neff et al., 1998). However, in the present study, fetal blood hematocrit and hemoglobin concentrations were not changed, and fetal plasma osmolality and the electrolyte concentrations also were unchanged in response to maternal infusion of nicotine. Furthermore, fetal urine volume, electrolytes, and osmolality were not affected too. Those data did not support the possibility

that nicotine-mediated fetal cardiovascular changes may be caused by vascular volume changes under the experimental condition.

In conclusion, maternal intravenous administration of low doses of nicotine significantly reduced fetal blood pO₂ and increased fetal arterial blood pressure in association of neural activation in the fetal brain. This provided new evidence that even exposure to low levels of nicotine during pregnancy could affect not only fetal cardiovascular systems and oxygen status, but also the fetal brain areas that are critical in the control of blood pressure. Notably, this was the first to show that maternal nicotine could increase cellular activation in the SFO and PVN in the fetal brain, adding new knowledge in nicotine-induced neurotoxic changes in the fetus. Although low doses of nicotine did not produce changes in maternal blood gases and cardiovascular systems, they induced significant responses *in utero*, indicating that fetuses were more sensitive and vulnerable to nicotine from environment. Importantly, the present study demonstrated that even exposure to low levels of nicotine could cause *in utero* hypoxia associated with pressor responses and over-activation of the brain cells in the fetus. This strongly suggests a high risk of second-hand smoking to babies inside women. The new information gained in the present study is important to preventive medicine.

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Fig. 1.
The effect of maternal intravenous nicotine on maternal arterial blood pO₂ (A), pCO₂ (B), and SO₂ (%) (C).

Fig. 2.
The effect of maternal intravenous nicotine on fetal arterial blood pO₂ (A), pCO₂ (B), and SO₂ (%) (C). vs. baseline value: #, $p < 0.05$; *, $p < 0.01$.

Fig. 3.

The effect of maternal intravenous nicotine on fetal arterial blood pressure. A, systolic blood pressure (SBP); B, diastolic blood pressure (DBP); C, mean arterial pressure (MAP). D, heart rate.

vs. baseline value: #, $p < 0.05$; *, $p < 0.01$.

Fig. 4. Number of total Fos immunoreactivity cells in the fetal paraventricular nuclei (PVN) and subfornic organ (SFO).

Fig. 5. Fos induced by maternal intravenous nicotine in the fetal paraventricular nuclei (PVN) (A and B) and the subfornic organ SFO (C and D). A and C, 0.9% NaCl; B and D, nicotine (25 µg/kg). ×20.

Table 1

Maternal arterial blood values.

Index	Baseline	15 min	30 min	90 min	120 min
Before and after 0.9%NaCl infusion					
pH	7.43 ± 0.01	7.43 ± 0.01	7.44 ± 0.03	7.43 ± 0.03	7.43 ± 0.02
Hb, g/dl	8.97 ± 0.57	8.83 ± 0.69	8.97 ± 0.59	9.00 ± 0.57	9.03 ± 0.54
Glu, mmol/l	3.68 ± 0.20	3.76 ± 0.04	3.65 ± 0.17	3.68 ± 0.13	3.69 ± 0.16
Lac, mmol/l	0.67 ± 0.12	0.63 ± 0.05	0.57 ± 0.05	0.57 ± 0.05	0.57 ± 0.05
Before and after 10 µg/kg nicotine infusion					
pH	7.45 ± 0.02	7.43 ± 0.04	7.43 ± 0.03	7.43 ± 0.06	7.42 ± 0.05
Hb, g/dl	9.15 ± 1.03	9.30 ± 0.96	9.07 ± 0.95	9.23 ± 1.23	9.21 ± 1.06
Glu, mmol/l	3.63 ± 0.27	3.52 ± 0.22	3.49 ± 0.25	3.50 ± 0.23	3.51 ± 0.40
Lac, mmol/l	0.64 ± 0.05	0.62 ± 0.07	0.60 ± 0.06	0.62 ± 0.07	0.60 ± 0.06
Before and after 25 µg/kg nicotine infusion					
pH	7.44 ± 0.03	7.44 ± 0.02	7.43 ± 0.03	7.44 ± 0.03	7.44 ± 0.03
Hb, g/dl	9.03 ± 0.83	8.90 ± 0.74	9.08 ± 0.77	9.13 ± 0.94	8.98 ± 1.00
Glu, mmol/l	3.69 ± 0.22	3.66 ± 0.23	3.64 ± 0.29	3.53 ± 0.27	3.61 ± 0.22
Lac, mmol/l	0.68 ± 0.07	0.65 ± 0.10	0.63 ± 0.07	0.63 ± 0.04	0.68 ± 0.07

Table 2

Maternal arterial blood electrolytes and osmolality.

Index	Baseline	15 min	30 min	90 min	120 min
Before and after 0.9%NaCl infusion					
Hct, %	28.00 ± 1.41	28.33 ± 0.47	28.00 ± 0.82	27.67 ± 0.47	27.67 ± 1.25
Na ⁺ , mmol/l	145.80 ± 1.44	145.63 ± 1.68	145.03 ± 2.47	146.47 ± 1.68	146.13 ± 1.77
K ⁺ , mmol/l	3.78 ± 0.13	3.71 ± 0.15	3.67 ± 0.11	3.78 ± 0.05	3.73 ± 0.10
Cl ⁻ , mmol/l	110.67 ± 3.44	108.50 ± 2.66	108.90 ± 1.91	111.50 ± 3.47	110.80 ± 4.10
Osm, mOsm/kg	297.00 ± 4.97	296.67 ± 3.86	297.67 ± 3.68	298.33 ± 5.91	297.67 ± 5.56
Before and after 10 µg/kg nicotine infusion					
Hct, %	29.17 ± 1.17	29.33 ± 1.63	29.00 ± 1.55	29.17 ± 1.33	29.00 ± 1.79
Na ⁺ , mmol/l	145.58 ± 1.77	145.28 ± 2.42	145.18 ± 2.45	146.48 ± 2.51	146.53 ± 2.64
K ⁺ , mmol/l	3.73 ± 0.25	3.78 ± 0.25	3.80 ± 0.24	3.73 ± 0.34	3.80 ± 0.36
Cl ⁻ , mmol/l	110.98 ± 1.51	108.67 ± 1.74	108.70 ± 4.44	108.80 ± 3.34	109.52 ± 3.50
Osm, mOsm/kg	297.40 ± 3.93	296.60 ± 1.36	298.80 ± 3.06	298.80 ± 3.12	297.20 ± 2.14
Before and after 25 µg/kg nicotine infusion					
Hct, %	28.67 ± 2.58	28.33 ± 2.25	28.83 ± 2.32	29.17 ± 2.48	28.67 ± 2.25
Na ⁺ , mmol/l	145.53 ± 1.36	146.02 ± 1.34	146.07 ± 1.25	146.32 ± 1.68	146.37 ± 1.25
K ⁺ , mmol/l	3.77 ± 0.12	3.76 ± 0.12	3.80 ± 0.05	3.79 ± 0.10	3.78 ± 0.16
Cl ⁻ , mmol/l	111.92 ± 3.55	112.73 ± 3.15	112.52 ± 2.84	111.67 ± 1.65	110.60 ± 2.30
Osm, mOsm/kg	297.00 ± 5.02	299.20 ± 6.46	295.80 ± 5.08	297.60 ± 9.77	298.80 ± 6.05

Table 3

Fetal arterial blood values.

Index	Baseline	15 min	30 min	90 min	120 min
Before and after 0.9%NaCl infusion					
pH	7.43 ± 0.01	7.44 ± 0.01	7.44 ± 0.01	7.44 ± 0.00	7.44 ± 0.01
Hb, g/dl	8.80 ± 0.08	8.77 ± 0.54	8.73 ± 0.29	8.60 ± 0.28	8.77 ± 0.05
Glu, mmol/l	1.69 ± 0.03	1.66 ± 0.04	1.68 ± 0.05	1.67 ± 0.03	1.68 ± 0.04
Lac, mmol/l	1.67 ± 0.05	1.67 ± 0.09	1.63 ± 0.05	1.60 ± 0.08	1.63 ± 0.05
Before and after 10 µg/kg nicotine infusion					
pH	7.43 ± 0.01	7.43 ± 0.01	7.43 ± 0.01	7.42 ± 0.01	7.42 ± 0.01
Hb, g/dl	8.62 ± 0.74	8.70 ± 0.65	8.68 ± 0.54	8.68 ± 0.66	8.68 ± 0.70
Glu, mmol/l	1.70 ± 0.10	1.66 ± 0.07	1.65 ± 0.11	1.61 ± 0.06	1.61 ± 0.05
Lac, mmol/l	1.50 ± 0.21	1.54 ± 0.19	1.56 ± 0.29	1.56 ± 0.27	1.58 ± 0.32
Before and after 25 µg/kg nicotine infusion					
pH	7.44 ± 0.02	7.43 ± 0.03	7.43 ± 0.02	7.43 ± 0.06	7.42 ± 0.03
Hb, g/dl	8.67 ± 0.61	8.60 ± 0.84	8.67 ± 0.75	8.72 ± 0.86	8.78 ± 0.82
Glu, mmol/l	1.60 ± 0.05	1.60 ± 0.03	1.59 ± 0.03	1.63 ± 0.08	1.61 ± 0.07
Lac, mmol/l	1.52 ± 0.11	1.56 ± 0.18	1.58 ± 0.11	1.52 ± 0.04	1.56 ± 0.11

Table 4

Fetal arterial blood electrolytes and osmolality.

Index	Baseline	15 min	30 min	90 min	120 min
Before and after 0.9%NaCl infusion					
Hct, %	27.00 ± 1.63	27.33 ± 0.94	27.33 ± 0.47	27.00 ± 0.00	27.33 ± 0.47
Na ⁺ , mmol/l	139.20 ± 1.81	139.00 ± 1.85	139.17 ± 1.88	139.70 ± 2.69	139.43 ± 2.53
K ⁺ , mmol/l	3.88 ± 0.05	3.66 ± 0.31	3.74 ± 0.07	3.80 ± 0.14	3.78 ± 0.12
Cl ⁻ , mmol/l	102.83 ± 4.57	103.73 ± 3.65	102.27 ± 2.35	105.00 ± 3.69	104.07 ± 3.69
Osm, mOsm/kg	290.67 ± 0.47	291.33 ± 1.89	292.33 ± 0.94	292.33 ± 3.77	292.00 ± 5.72
Before and after 10 µg/kg nicotine infusion					
Hct, %	27.00 ± 0.89	27.20 ± 1.33	27.80 ± 1.47	27.20 ± 1.72	27.60 ± 0.80
Na ⁺ , mmol/l	139.47 ± 2.09	140.03 ± 1.68	139.95 ± 2.09	139.95 ± 1.89	139.45 ± 1.74
K ⁺ , mmol/l	3.90 ± 0.12	3.90 ± 0.18	3.83 ± 0.12	3.87 ± 0.14	3.81 ± 0.16
Cl ⁻ , mmol/l	103.97 ± 2.34	103.95 ± 2.60	105.27 ± 2.59	105.17 ± 2.03	104.82 ± 1.77
Osm, mOsm/kg	291.17 ± 6.18	292.50 ± 5.09	294.33 ± 3.39	293.67 ± 4.13	290.50 ± 5.47
Before and after 25 µg/kg nicotine infusion					
Hct, %	27.00 ± 1.41	27.00 ± 1.55	27.00 ± 1.41	27.17 ± 1.60	27.00 ± 1.26
Na ⁺ , mmol/l	139.25 ± 2.02	139.20 ± 2.55	139.57 ± 2.61	139.95 ± 2.64	140.17 ± 2.48
K ⁺ , mmol/l	3.72 ± 0.14	3.69 ± 0.21	3.66 ± 0.20	3.72 ± 0.16	3.75 ± 0.19
Cl ⁻ , mmol/l	106.15 ± 5.26	105.32 ± 4.18	103.67 ± 2.67	103.62 ± 3.77	104.65 ± 0.98
Osm, mOsm/kg	291.17 ± 5.12	291.33 ± 5.43	291.50 ± 4.72	294.00 ± 8.92	292.83 ± 9.22

Table 5

Fetal urine values in interval 30 min before and after nicotine infusion.

Index	Pre30-0 min	0-30 min	30-60 min	60-90 min	90-120 min
Before and after 10 µg/kg nicotine infusion					
Na ⁺ , mmol/l	25.57 ± 1.27	24.22 ± 1.78	25.42 ± 3.46	27.38 ± 3.32	22.92 ± 1.99
K ⁺ , mmol/l	4.87 ± 0.31	4.76 ± 0.18	4.70 ± 0.32	5.27 ± 0.70	4.69 ± 0.29
Cl ⁻ , mmol/l	19.88 ± 0.98	17.77 ± 2.75	19.11 ± 3.62	18.07 ± 2.78	19.26 ± 2.97
Ca ⁺⁺ , mmol/l	1.62 ± 0.13	1.58 ± 0.14	1.59 ± 0.15	1.56 ± 0.26	1.57 ± 0.14
Osm, Osm/kg	130.50 ± 2.70	128.89 ± 7.01	129.33 ± 6.56	134.33 ± 7.77	129.78 ± 6.37
pH	6.70 ± 0.29	6.62 ± 0.27	6.64 ± 0.24	6.58 ± 0.37	6.58 ± 0.22
Volume, ml	16.43 ± 1.62	14.83 ± 1.90	15.10 ± 2.60	14.53 ± 2.57	16.50 ± 2.58
Before and after 25 µg/kg nicotine infusion					
Na ⁺ , mmol/l	25.50 ± 1.85	26.19 ± 2.43	27.51 ± 4.69	28.53 ± 1.66	28.37 ± 0.69
K ⁺ , mmol/l	4.58 ± 0.09	4.91 ± 0.54	4.41 ± 0.10	4.93 ± 0.23	4.56 ± 0.13
Cl ⁻ , mmol/l	20.65 ± 2.44	23.72 ± 4.66	21.82 ± 2.92	21.88 ± 3.21	21.80 ± 1.17
Ca ⁺⁺ , mmol/l	1.67 ± 0.09	1.55 ± 0.14	1.62 ± 0.09	1.55 ± 0.14	1.57 ± 0.09
Osm, Osm/kg	127.83 ± 6.21	133.44 ± 5.97	132.00 ± 7.43	136.00 ± 6.47	130.78 ± 7.15
pH	6.78 ± 0.16	6.78 ± 0.06	6.86 ± 0.04	6.70 ± 0.06	6.81 ± 0.10
Volume, ml	16.08 ± 1.09	17.37 ± 1.11	16.00 ± 1.72	16.37 ± 1.13	17.70 ± 2.35

Table 6

Measurement of maternal arterial blood pressure and heart rate after intravenous administration of 25 µg/kg nicotine.

	Baseline	15 min	30 min	90 min	120 min
Before and after 0.9% NaCl infusion					
SBP, mmHg	120.3 ± 4.1	119.0 ± 5.7	119.6 ± 2.8	122.3 ± 5.0	123.7 ± 4.0
DBP, mmHg	79.4 ± 3.5	77.8 ± 7.2	78.8 ± 6.9	80.3 ± 4.4	80.2 ± 5.3
MAP, mmHg	98.9 ± 2.4	97.7 ± 5.7	96.4 ± 6.5	98.6 ± 2.4	96.6 ± 4.5
HR, bpm	106.5 ± 6.9	109.3 ± 10.7	108.6 ± 12.0	106.7 ± 6.1	105.3 ± 4.1
Before and after 10 µg/kg nicotine infusion					
SBP, mmHg	120.2 ± 4.2	121.1 ± 4.3	122.8 ± 7.2	122.4 ± 7.6	121.3 ± 6.1
DBP, mmHg	79.1 ± 6.6	78.2 ± 8.2	80.5 ± 12	81.3 ± 10.9	80.9 ± 11.6
MAP, mmHg	97.8 ± 5.1	97.4 ± 5.5	99.2 ± 7.1	100.3 ± 7.5	101.8 ± 5.2
HR, bpm	108.1 ± 12.3	109.6 ± 11.0	106.9 ± 17.0	106.1 ± 9.6	108.4 ± 11.8
Before and after 25 µg/kg nicotine infusion					
SBP, mmHg	122.1 ± 4.5	122.1 ± 4.9	123.0 ± 5.4	122.9 ± 7.8	122.1 ± 8.6
DBP, mmHg	79.2 ± 5.2	78.7 ± 5.1	79.7 ± 5.6	81.0 ± 6.5	80.5 ± 6.6
MAP, mmHg	97.8 ± 4.7	97.6 ± 4.2	95.8 ± 5.0	98.8 ± 5.4	96.2 ± 6.3
HR, bpm	105.4 ± 4.0	105.8 ± 4.2	110.6 ± 7.8	109.9 ± 10.6	109.3 ± 10.9