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Maternal smoking during pregnancy may impair pulmonary function in infants and children but the exact mechanisms underlying these changes remain to be determined.

Timed pregnant Sprague-Dawley rats were injected subcutaneously with nicotine at a dose of 2 mg/kg/day from day 3 to day 21 of gestation. A control group was injected with saline.

Nicotine-exposed rats exhibited a significantly higher lung volume and lung volume/body weight ratio on postnatal day 28 when compared with control rats. Lung saturated phosphatidylcholine contents and expressions of surfactant protein (SP)-A, SP-B, SP-C, and SP-D mRNAs were similar between controls and nicotine-exposed rats. On postnatal day 1, nicotine-exposed rats exhibited a significantly lower volume fraction of alveolar airspace, higher volume fraction of alveolar wall, larger alveolar wall thickness, and smaller surface density of alveolar walls. On postnatal day 28, nicotine-exposed rats exhibited a significantly smaller alveolar wall thickness and larger surface density of alveolar walls.

In conclusion, maternal nicotine exposure does not affect lung surfactant lipid or surfactant protein genes expression but does alter lung development in postnatal rats. These results suggest that exposure to antenatal nicotine may produce structural alterations in the developing respiratory system and create various changes during different stages of lung development.

Keywords: Nicotine, saturated phosphatidylcholine, surfactant protein, lung

Introduction

Maternal smoking has been associated with pregnancy complications including fetal growth retardation, fetal and neonatal death, a higher incidence of spontaneous abortion, and premature delivery [1]. Nicotine is the causative agent for these effects because it is a major pharmacological constituent of tobacco smoke that readily crosses the placenta and is concentrated in the fetus at a

level 15% higher than that in the mother [2]. Animal studies have demonstrated that maternal nicotine and tobacco smoke exposure adversely affect fetal growth, fetal lung development, and pulmonary function at birth as manifested by decreased expiratory flow rates and increased pulmonary resistance [3-5].

The incidence of respiratory distress syndrome for infants of mothers who smoke during pregnancy was lower than that of infants of nonsmoking mothers [6, 7]. Smoke-exposed fetuses have higher amniotic fluid saturated phosphatidylcholine levels and lecithin-to-sphingomyelin ratios than unexposed fetuses [8]. Although maternal smoking during pregnancy enhances fetal lung maturation, several studies have demonstrated the negative impact of prenatal maternal smoking on early infant lung function [9-11]. Hanrahan et al. found that forced expiratory flow rates in infants born to smoking mothers was significantly lower than those found in infants whose mothers did not smoke during pregnancy [10]. Milner et al. performed lung function tests within 72 h of delivery and found that maternal smoking in pregnancy reduced static compliance in boys and conductance in girls [11]. These studies suggested that maternal smoking during pregnancy might impair *in utero* airway development, although the exact mechanisms underlying these changes remain to be determined. Maritz et al. reported that prenatal and neonatal nicotine exposure might decrease elastin content and arrest alveolar development in neonatal rats [12, 13]. Sekhon et al. found that nicotine exposure during pregnancy not only caused lung hypoplasia but it also increased airway wall thickness and stimulated the synthesis of collagen in airway wall and alveolar compartments in preterm monkeys [14, 15]. The aim of this study was to elucidate the mechanism underlying the impaired lung function in infancy and childhood.

Methods

Animals

Timed pregnant Sprague-Dawley rats were injected subcutaneously with nicotine tartrate (Sigma, St. Louis, MO, USA) at a dose of 2 mg/kg/day from day 3 to day 21 of gestation. A control group was injected with an equal volume of 0.9% NaCl. On days 1, 7, 14, 21, 28, 35, and 42 after birth, rats were randomly selected from each group, irrespectively of sex.

Measurement of lung tissue saturated phosphatidylcholine

Right lung was homogenized and extracted with chloroform-methanol [16]. Lipid extracts were treated with osmium tetroxide, and saturated phosphatidylcholine was recovered by alumina column chromatography and was quantified by phosphorus assay [17, 18].

Surfactant protein expression by RT-PCR

Gene expressions of SP-A, SP-B, SP-C, and SP-D were measured with reverse transcriptase-polymerase chain reaction (RT-PCR) in right lung.

Morphological analysis of lung

Left lung was fixed by 10% buffered formalin at a pressure of 25 cmH_2O for 10 min. After ligation of the left main bronchus, the lungs were placed in buffered formalin for 24 h to continue fixation. Left lung volume was determined by fluid displacement [19] and extrapolated to obtain total lung volume using total lung weight. Lung morphometry examined by point counting using a light microscope showed that the proportion of parenchyma was highly

constant in all lobes [20]. Digitized images from 20 non-overlapping fields were captured from lung sections. Images were printed and examined at a final magnification of ×400. The number of points that fell on alveolar airspace, alveolar wall, alveolar duct, and nonparenchyma were counted by superimposing transparent grids (49 points) onto enlarged printed images [21].

Statistical analysis

Data are expressed as the mean ± SEM. Between-group comparisons were made using Student's *t*-test. Differences were considered significant at $p < 0.05$.

Results

There were 110 fetuses from 11 rats in the control group and 101 fetuses from 12 rats in the nicotine-treated group. There was no significant difference in litter size between the two study groups.

Effects of antenatal nicotine treatment on maternal body weight

Body weights before treatment were comparable between control and nicotine-treated dams (Figure 1). Body weights of nicotine-treated dams were lower than those of control dams from gestational days 5 to 21, and the values reached statistical significance on gestational days 17, 20, and 21.

Body weight, lung weight, and lung volume in control and nicotine-exposed rats

Effects of maternal nicotine exposure on neonatal body weight, lung weight, and lung volume are presented in Table 1.

Saturated phosphatidylcholine in control and nicotine-exposed rat lung

Saturated phosphatidylcholine per body weight was significantly higher in total lung on postnatal day 1 than at any other age in both groups ($p < 0.001$, Figure 2).

Surfactant protein gene expression in control and nicotine-exposed rat lung

Lung SP-A, SP-B, SP-C, and SP-D mRNAs expressions were similar between control and nicotine-exposed rats during the study period (Figure 3).

Effects of maternal nicotine exposure on lung morphometry

On postnatal day 1, nicotine-exposed rats exhibited a significantly lower volume fraction of alveolar airspace and a significantly higher volume fraction of alveolar wall than did control rats (Figure 4).

Discussion

In this study, we found that total lung saturated phosphatidylcholine content was higher on postnatal day 1 than at any other age and fell as the rats aged in both study groups. These results are in agreement with the generalization that total lung surfactant pools are higher at term birth than at any other time in an animal's life [23]. Total saturated phosphatidylcholine contents were similar between control and nicotine-exposed rat lungs on each postnatal day. The effects of cigarette smoking on surfactant status are inconsistent. Human fetuses exposed to intrauterine cigarette smoke have higher amniotic fluid saturated phosphatidylcholine levels than do unexposed fetuses [8]. Wirtz et al. noted that cigarette smoke directly inhibits phosphatidylcholine secretion in cultured rat alveolar type II cells [24]. As surfactant lipid was not measured separately in lung tissue or alveolar wash we do not know the exact effect of nicotine administration on the secretion of

surfactant in the rat lung. Maternal nicotine exposure during pregnancy and lactation has been reported to increase the type II cell count and lamellar body content of type II cells in early postnatal rats [25]. However, in this study, we found that prenatal nicotine exposure had no effect on total lung saturated phosphatidylcholine content in postnatal rats. These results suggest that maternal nicotine exposure might alter the relationship between volume density of type II cells and surfactant content in lung tissue. Similar phenomena have been observed in fetal guinea pig lungs during prenatal starvation and in fetal lamb lungs with an experimental diaphragmatic hernia [26, 27].

Four lung-specific proteins have been found to be associated with the surfactant [28]. Surfactant proteins are synthesized primarily by alveolar type II cells or bronchiolar epithelial cells and are required both for the transition between lamellar bodies and tubular myelin, and for the spreading of tubular myelin components to the surface film [29]. Schellhase et al. investigated the ontogeny of SP-A, SP-B, and SP-C mRNAs in the developing rat lung and speculated that genes for surfactant proteins may be differentially regulated [30]. In this study, we describe for the first time the ontogeny of surfactant protein mRNAs in the postnatal rat lung after antenatal nicotine exposure. The results show that maternal nicotine exposure did not affect the gene expressions of SP-A, SP-B, SP-C, and SP-D in the lung and suggest that the pulmonary epithelium is not affected by *in utero* exposure to nicotine. These findings are in agreement with the observations of Hermans et al. [31], who found the absence of change in SP-A in amniotic fluid at term and suggested that maternal smoking during pregnancy is not associated with alterations in the secretory functions of the epithelium of the distal airway or the alveoli.

In the present study we found that nicotine-exposed rats exhibited a significantly greater alveolar wall thickness and smaller surface density of the alveolar walls on postnatal day 1. Decreased alveolar surface area is associated with decreased lung compliance and increased resistance while increased alveolar wall thickness is associated with disturbed pulmonary diffusion capacity. Sekhon et al. found that nicotine exposure during pregnancy increased airway wall area and stimulated the synthesis and accumulation of collagen types I and III in airway and alveolar walls during the fetal period [15]. We speculated that the increased alveolar wall thickness of nicotine-exposed rats on postnatal day 1 in our study was caused by collagen deposition. This hypothesis needs further study for confirmation. Changes in lung volume and surface density of alveolar walls in control rats during the first 21 days of life were similar to those reported by Burri et al. [32]. On postnatal day 28, nicotine-exposed rats exhibited an increased volume fraction of alveolar airspace, thinner alveolar walls, and a larger surface density of alveolar walls. These results are in agreement with the findings of Nikula et al. [33], who found that mice exposed to cigarette smoke for 7 to 13 months had significantly greater lung volumes and larger alveoli.

In conclusion, this study found that maternal nicotine exposure during pregnancy does not influence lung surfactant lipid or surfactant protein genes expression but does alter lung development in postnatal rats. These results suggest that exposure to antenatal nicotine may produce structural alterations in the developing respiratory system and create various changes during different stages of lung development.

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Table 1. Effect of antenatal nicotine exposure on body weight, lung weight, and lung volume

	n	Age (d)	Body weight (g)	Lung weight (g)	Lung volume (ml)
Control	16	1	7.42 ± 0.24	0.13 ± 0.00	0.50 ± 0.03
Nicotine	17	1	7.16 ± 0.15	0.13 ± 0.01	0.40 ± 0.04
Control	14	7	16.25 ± 0.80	0.27 ± 0.02	1.05 ± 0.03
Nicotine	15	7	16.07 ± 0.71	0.27 ± 0.02	0.93 ± 0.06
Control	15	14	34.87 ± 1.47	0.54 ± 0.02	1.39 ± 0.04

Nicotine	15	14	34.80 ± 0.94	0.53 ± 0.02	1.47 ± 0.17
Control	19	21	62.11 ± 1.73	0.54 ± 0.01	1.82 ± 0.11
Nicotine	13	21	60.54 ± 1.24	0.58 ± 0.02	1.83 ± 0.09
Control	15	28	95.40 ± 2.86	0.70 ± 0.03	2.21 ± 0.13
Nicotine	14	28	96.43 ± 2.81	0.76 ± 0.03	2.90 ± 0.19*
Control	16	35	157.56 ± 3.93	1.05 ± 0.05	2.54 ± 0.11
Nicotine	14	35	151.64 ± 3.17	1.04 ± 0.03	2.50 ± 0.09
Control	15	42	199.07 ± 4.90	1.21 ± 0.03	4.31 ± 0.09
Nicotine	13	42	203.69 ± 6.22	1.21 ± 0.07	3.62 ± 0.08

Values are the mean ± SEM; *p < 0.05 compared with control rats at each time point.

Figure 1

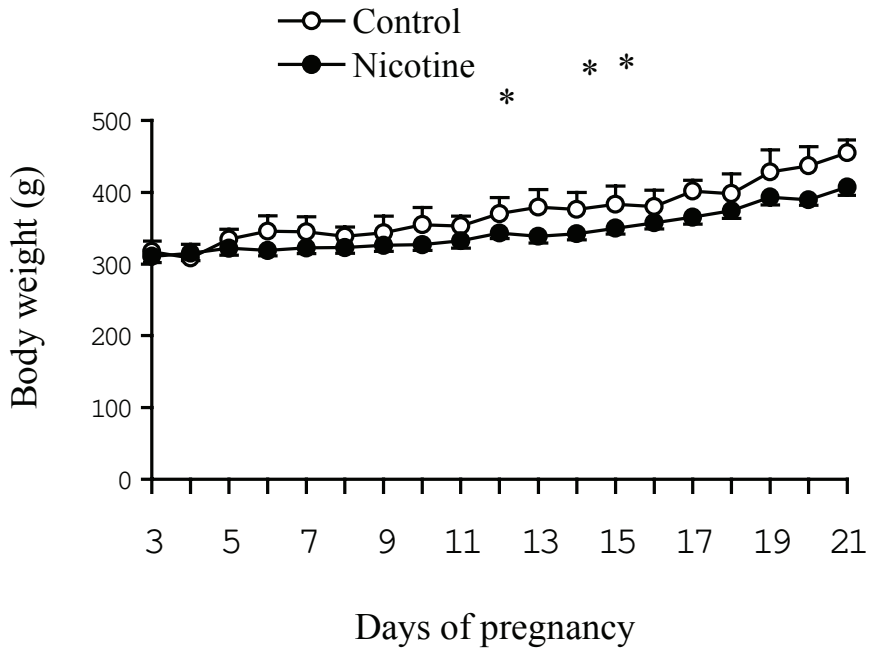


Figure 2

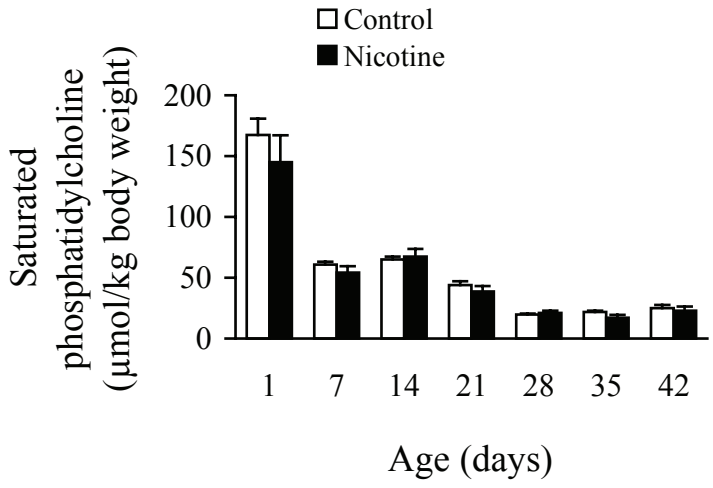


Figure 3

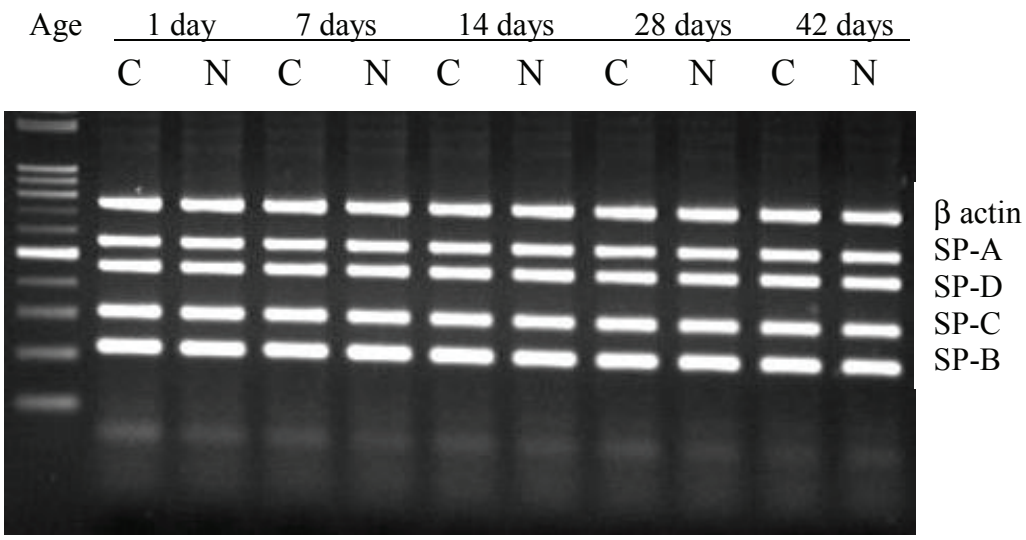


Figure 4

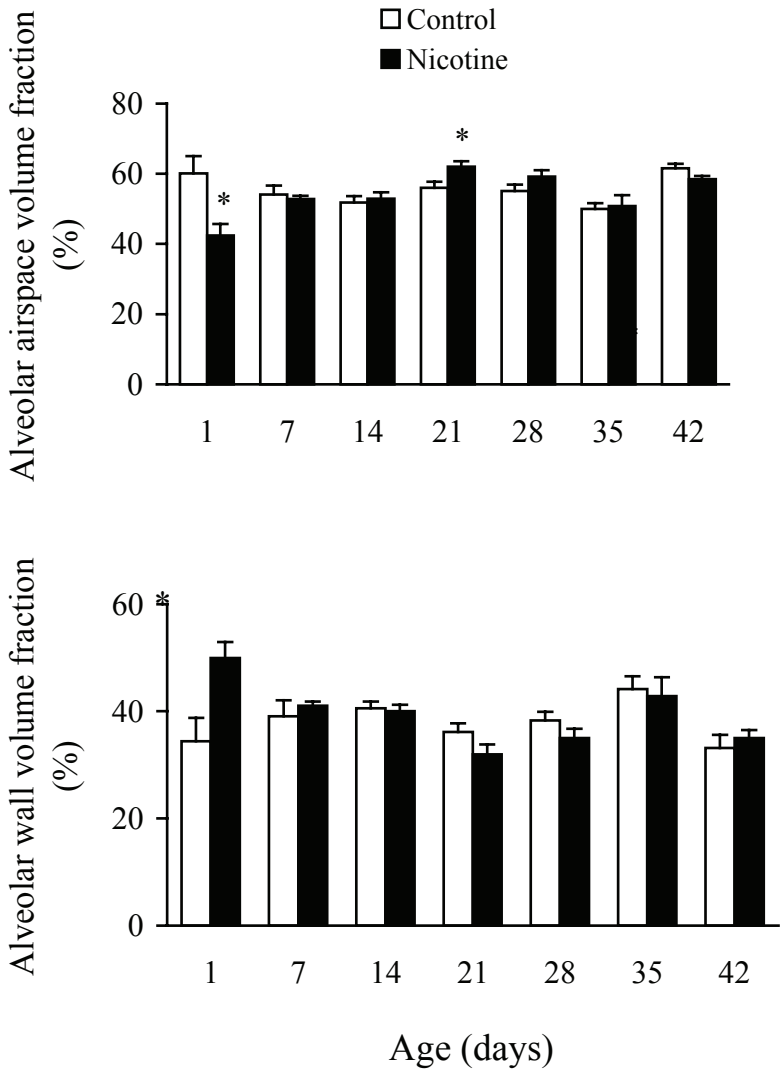
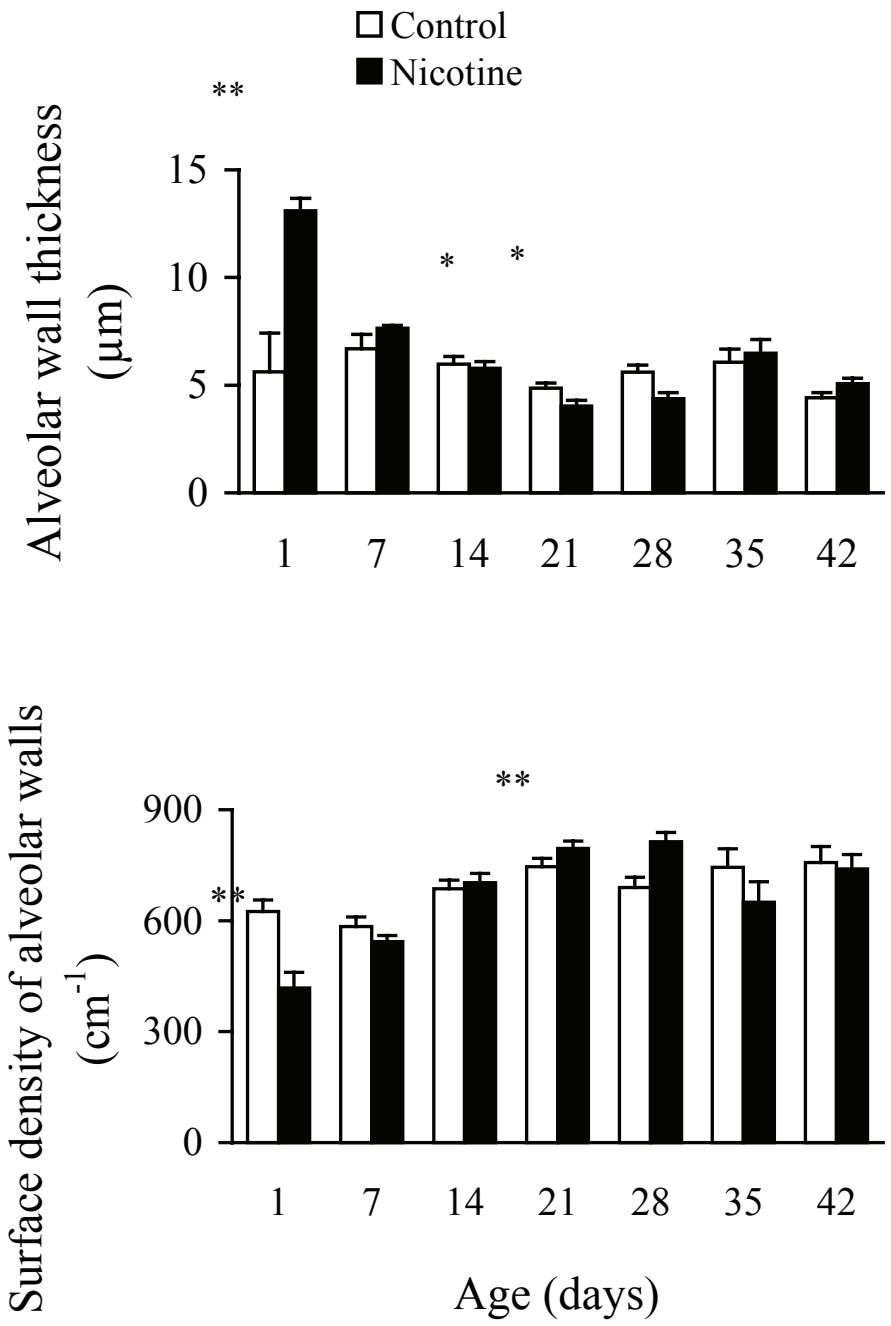


Figure 5


Control
Nicotine
Alveolar wall thickness (μm)
15
10
5
0
**
*
*
1
7
14
21
28
35
42
Surface density of alveolar walls (cm-1)
900
600
300
0
**
**
1
7
14
21
28
35
42
Age (days)