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Effect of prenatal exposure to cigarette smoke on the conduction of sensory nerve fibers in the rat

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Aim: To investigate the effect of prenatal exposure to cigarette smoke (CS) on the conduction of sensory nerve fibers in the adult rat offspring.

Materials and methods: Pregnant rats were exposed to CS by inhalation during the gestation period. The saphenous nerve in the adult offspring was exposed in vivo, and the conduction parameters of the threshold, maximum strength of stimulation, conduction velocity, and amplitude of compound action potentials (CAPs) of Aαβ-, Aδ-, and C-fibers were examined.

Results: Prenatal exposure to CS induced significant reduction ($P < 0.005$) in the peak amplitude of CAPs of Aαβ- and Aδ-fibers. The conduction velocity of Aαβ-fibers and C-fibers was significantly ($P < 0.025$-0.05) increased after prenatal exposure to CS.

Conclusion: Prenatal exposure to CS attenuates the conduction of sensory nerve fibers in the rat saphenous nerve.

Key words: Cigarette smoke, sensory nerve fibers, compound action potentials, pregnancy

Introduction

Maternal cigarette smoking during pregnancy has been associated with many harmful effects on the fetus, including low birth weight, preterm delivery, and sudden infant death syndrome (1-5). In addition, cigarette smoke (CS) exposure during fetal development induces severe neurocognitive deficits and neurobehavioral disorders (6,7). Additional risks increase with prenatal exposure to CS, such as lung airway alterations in structure and function (8-10), cardiovascular dysfunctions (11-13), and nervous system deficits (14-16). It has been shown that CS contains a complex mixture of many toxic compounds, including nicotine, cotinine, and carbon monoxide (17,18).

Animal studies have indicated that prenatal exposure to CS or nicotine administration during pregnancy induced severe fetal effects, including reduced body weight (19,20), liver damage (21,22), pulmonary morphological and functional alterations (23-25), kidney weight loss (26), cardiovascular dysfunction (27,28), and nervous system deficits (29). The present study aimed to examine the effect of prenatal exposure of rats to CS on the conduction of the peripheral sensory nerve fibers.

Materials and methods

Maternal CS exposure

Sprague-Dawley rats weighing 200-250 g were used in this study. Housed together overnight were 2 virgin females and 1 male. The next morning, vaginal smears were examined and the presence of sperm was taken to indicate pregnancy. After mating, the male was removed and the females were individually

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caged and exposed to CS via whole-body inhalation 4 times daily (15 min each) during the full gestational period. The smoke was crudely generated from the burning of cigarettes (Marlboro) by pumping, which regularly drew smoke (4 puffs/min) to the rats in a glass container. This is a rather low dose of CS, roughly equivalent to smoking less than a pack of cigarettes/day. Under these exposure conditions, the pregnant rats were normal and gained approximately the same amount of weight during the gestation period. The room temperature was maintained at 25-28 °C, and the rats were provided food and tap water ad libitum, except during inhalation exposures. The pups were left to grow until the final acute electrophysiological experiments.

Animal preparation

Acute experiments were carried out on 15 male rat offspring (200-250 g) that had been prenatally exposed to CS. A control group of 10 male rats of similar age and weight were used for comparison. The animals were deeply anesthetized with urethane (1.5 g/kg, intraperitoneally), a tracheal cannula was inserted, and body temperature was maintained at around 37 °C with a heat blanket system (Harvard Apparatus, Harvard Bioscience Inc., Holliston, MA, USA) placed under the animal, which was automatically controlled by a small rectal thermistor probe. Blood pressure was monitored via the cannulated left common carotid artery, and systemic blood pressure was 80-120 mmHg. The fur on the medial aspect of the right thigh was clipped, the skin was opened, and the saphenous nerve was carefully exposed for recording and stimulation. For electrical stimulation, a small segment (5 mm) of the nerve was gently dissected from the connective tissue at a distal site above the knee and placed on a pair of bipolar platinum wire hook electrodes. For the recording of the compound action potentials (CAPs), the nerve was gently exposed in the upper thigh, cut, and placed on a similar pair of platinum recording electrodes. The recording electrodes were connected to an amplifier with a band width of 5-5000 Hz (Digitimer, Hertfordshire, UK). The nerve was covered by a pool of warm mineral oil made from skin flaps sutured to a fixed metal ring. Electrical stimulation of the A-fibers was done at 0.2-0.3 mA with a 0.05-ms pulse duration and 10-Hz frequency, while the C-fibers were stimulated at 1.75-1.85 mA with a 0.5-ms pulse duration and 1 Hz-frequency. The recording signals of the CAPs were amplified, monitored by a loudspeaker, displayed on a Tektronix 2232 digital storage oscilloscope (Tektronix, Beaverton, OR, USA), and stored in a computer using a software program (LabVIEW, National Instruments Corp., Austin, TX, USA). Conduction distances between recording and stimulating electrodes were 20-25 mm. Conduction velocities of the nerve fibers were determined by measuring the latencies from the onset of stimulation to the appearance of the first component of the CAPs.

Statistical analysis

Statistical analysis was performed using an unpaired t-test between the means of the prenatal CS exposure group and controls using Excel software. Differences were considered significant at $P < 0.05$. Values are expressed as mean ± standard error (SE).

Results

Effect of prenatal exposure to CS on the CAPs of Aαβ-fibers

The peak amplitude of the CAPs of Aαβ-fibers in the rat saphenous nerve after prenatal exposure to CS was reduced compared to that of the controls (Figure 1).

The means of the threshold, maximum strength of stimulation, conduction velocity, and peak amplitude of the CAPs of Aαβ-fibers of the rat saphenous nerve in the controls and after prenatal exposure to CS are shown in Table 1.

The mean peak amplitude of the CAPs of Aαβ-fibers after prenatal exposure to CS was significantly reduced ($P < 0.005$) compared to the controls. In contrast, the conduction velocity of Aαβ-fibers after prenatal exposure to CS was significantly increased ($P < 0.025$) compared to the controls. The threshold and maximum strength of stimulation of Aαβ-fibers after prenatal exposure to CS were not significantly

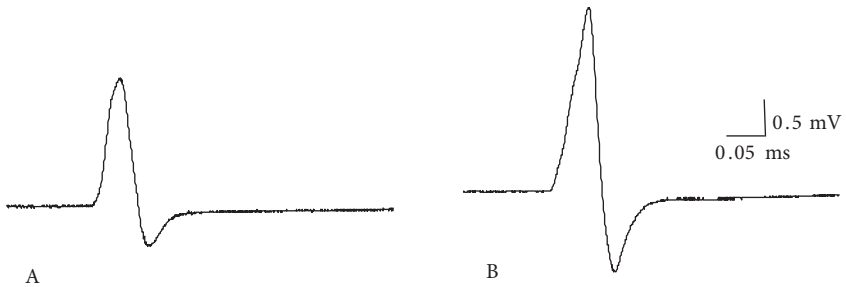

Figure 1. CAPs of Aαβ-fibers of the rat saphenous nerve after prenatal exposure to CS (A) and in the controls (B). Note: same scale for both traces.

Table 1. Means of the threshold, maximum strength of stimulation, conduction velocity, and peak amplitude of the CAPs of Aαβ-fibers of the rat saphenous nerve in the controls and after prenatal exposure to CS.

Groups	Threshold (mA)	Maximum (mA)	Conduction velocity (m/s)	Amplitude (mV)
CS exposure (n = 15)	0.22 ± 0.01	0.27 ± 0.01	51.88 ± 1.15	1.83 ± 0.1
Controls (n = 10)	0.2 ± 0.01	0.24 ± 0.01	46.65 ± 2.39	2.48 ± 0.18
P*	0.1	0.25	0.025*	0.005*

different from those of the controls (P > 0.1 and P > 0.25, respectively).

Effect of prenatal exposure to CS on the CAPs of Aδ-fibers

The peak-to-peak amplitude of the CAPs of Aδ-fibers in the rat saphenous nerve after prenatal exposure to CS was reduced compared to the controls (Figure 2).

The means of the threshold, maximum strength of stimulation, conduction velocity, and peak-to-peak amplitude of the CAPs of Aδ-fibers of the rat saphenous nerve in the controls and after prenatal exposure to CS are shown in Table 2.

The mean peak-to-peak amplitude of the CAPs of Aδ-fibers after prenatal exposure to CS was significantly reduced (P < 0.005) compared to the controls. However, the threshold, maximum strength of stimulation, and conduction velocity of the Aδ-fibers after prenatal exposure to CS were not significantly different (P > 1.0-0.25) from those of the controls.

Effect of prenatal exposure to CS on the CAPs of C-fibers

The peak-to-peak amplitude of the CAPs of C-fibers of the rat saphenous nerve was unaffected after prenatal exposure to CS (Figure 3).

The means of the threshold, maximum strength of stimulation, conduction velocity, and peak-to-peak amplitude of CAPs of C-fibers of the rat saphenous nerve in the controls and after prenatal exposure to CS are shown in Table 3.

The mean peak-to-peak amplitude of the CAPs of C-fibers after prenatal exposure to CS was not significantly different from that of the controls (P > 0.1). The threshold and conduction velocity of the C-fibers were significantly increased (P < 0.05) after prenatal exposure to CS compared to the controls. The maximum strength of stimulation after prenatal exposure to CS was not significantly different (P > 0.1) from that of the controls.

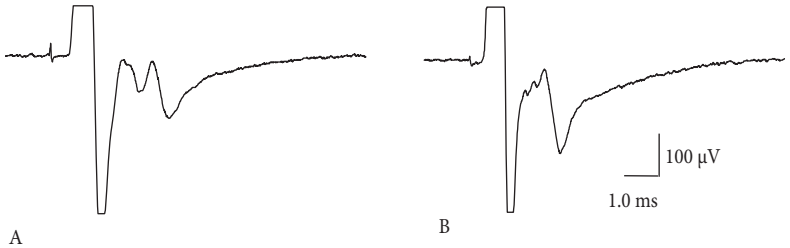

Figure 2. CAPs of Aδ-fibers of the rat saphenous nerve after prenatal exposure to CS (A) and controls (B). Note: same scale for both traces.

Table 2. Means of the threshold, maximum strength of stimulation, conduction velocity, and peak-to-peak amplitude of CAPs of Aδ-fibers of the rat saphenous nerve in the controls and after prenatal exposure to CS.

Groups	Threshold (mA)	Maximum (mA)	Conduction velocity (m/s)	Amplitude (µV)
CS exposure (n = 15)	0.28 ± 0.01	0.41 ± 0.03	11.73 ± 0.44	139.31 ± 12.14
Controls (n = 10)	0.24 ± 0.01	0.33 ± 0.02	12.00 ± 0.38	219.6 ± 18.89
P*	0.1	0.25	0.25	0.005*

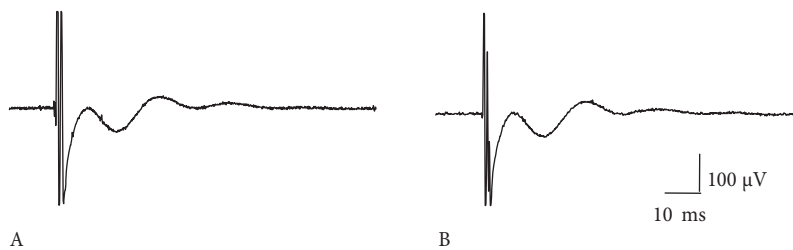

Figure 3. CAPs of C-fibers of the rat saphenous nerve after prenatal exposure to CS (A) and controls (B). Note: same scale for both traces.

Table 3. Means of the threshold, maximum strength of stimulation, conduction velocity, and peak-to-peak amplitude of CAPs of C-fibers of the rat saphenous nerve in the controls and after prenatal exposure to CS.

Groups	Threshold (mA)	Maximum (mA)	Conduction velocity (m/s)	Amplitude (µV)
CS exposure (n = 15)	1.81 ± 0.01	1.98 ± 0.04	1.32 ± 0.04	87.38 ± 7.0
Controls (n = 10)	1.77 ± 0.01	1.9 ± 0.02	1.20 ± 0.02	86.80 ± 4.76
P*	0.05*	0.1	0.05*	0.00

Discussion

The present study showed that prenatal exposure of the rats to CS induced significant reduction ($P < 0.005$) in the amplitude of the CAPs of myelinated Aαβ- and Aδ-nerve fibers. This attenuation in the conduction of the nerve fibers is probably due to either structural damage or functional block caused by CS components or hypoxia during fetal development. In the mouse, prenatal nicotine treatment induced a neuroteratogenic effect on the central nervous system (30) and caused neuronal death in some brain areas (31). In the rat, several studies confirmed the neurodegenerative action of prenatal nicotine administration on neurons in the medulla oblongata (32) and cerebral cortex (29).

Unlike the CAPs of myelinated Aαβ- and Aδ-fibers, the present results showed that the amplitude of the CAPs of unmyelinated C-fibers was not significantly affected ($P > 0.25$) by prenatal CS exposure. This selective effect of CS may indicate that the nicotinic type of nerve fibers in the rat saphenous nerve was the most likely to be affected by prenatal CS exposure, since it was found that the nicotinic acetylcholine receptors in the early embryonic mouse cerebral cortex were highly affected by the nicotine treatment (33).

It is known that tobacco smoke contains several highly toxic compounds such as nicotine, cotinine, and carbon monoxide (17,18), which can readily pass through the placenta, becoming highly concentrated in the fetal blood and amniotic fluid (3,34,35). The accumulation of these products in fetal tissues induces severe effects in metabolism and development (3,36,37). The brain is the most severely affected organ during the early embryonic developmental stages (38). Moreover, some compounds in CS act as vasoconstrictors to the uteroplacental arteries, which reduces blood flow (3,39), causing deprivation of oxygen and nutrient supply to the fetus. In conclusion, the results of the present study showed that prenatal CS exposure of the rats induces marked permanent attenuation in the conduction of sensory cutaneous nerve fibers, as demonstrated by significant reduction in the amplitude of the CAPs of Aαβ- and Aδ-nerve fibers and the conduction velocity of Aαβ-fibers. However, only slight nonsignificant changes were found in the conduction velocity, threshold, and maximum strength of stimulation of these nerve fibers. The unmyelinated C-fibers were affected by an increase in the threshold and conduction velocity, but not in the CAPs or in the maximum stimulation strength.

Acknowledgments

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