Physiol. Res. 49: 135-141, 2000

Passive Smoking Impairs Endothelium-Dependent Relaxation of Isolated Rabbit Arteries

J. TÖRÖK[1], A. GVOZDJÁKOVÁ[2], J. KUCHARSKÁ[2], I. BALAŽOVJECH[3], S. KYSELÁ[1], F. ŠIMKO[4], J. GVOZDJÁK[2]

[1]*Institute of Normal and Pathological Physiology Slovak Academy of Sciences,* [2]*Pharmacobiochemical Laboratory,* [3]*Second Department of Internal Medicine and* [4]*Department of Pathophysiology, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic*

Received August 15, 1999
Accepted September 21, 1999

Summary

The aim of the present study was to examine the effect of prolonged passive smoking (lasting 3 weeks) on plasma catecholamine levels and reactivity of isolated rabbit arteries. Plasma noradrenaline, adrenaline and dopamine levels were determined radioenzymatically. Isolated rings of the thoracic aorta and carotid artery were suspended in organ chambers and connected to a force transducer for the recording of isometric tension. Plasma noradrenaline levels were found to be significantly elevated in rabbits subjected to passive smoking for 3 weeks. Plasma adrenaline and dopamine levels were not changed. Transmural nerve stimulation of arterial rings evoked frequency-dependent contractions. Prolonged passive smoking did not affect neurogenic contractions of the arteries tested. On the other hand, endothelium-dependent relaxations of phenylephrine-precontracted arteries were significantly impaired. Furthermore, hypertrophy of the left ventricle was observed. In conclusion, passive smoking impairs endothelium-dependent relaxations but not neurogenic contractions of systemic arteries. The impaired relaxations of arteries may be, at least in part, mediated through the degradation of released nitric oxide by superoxide anions derived from cigarette smoke.

Key words

Passive smoking • Cardiac hypertrophy • Endothelium-dependent relaxation • Thoracic aorta • Carotid artery

Introduction

Long-term passive smoking causes considerable injury of the oxidative and phosphorylating function of myocardial mitochondria and may provoke smoke-induced mitochondrial cardiomyopathy (Gvozdjáková *et al.* 1984, 1995). In the peripheral vascular system, morphological abnormalities in endothelial cells (swelling, extensive subendothelial edema, increased number of subendothelial macrophages) have been observed in human subjects and animals exposed to cigarette smoke for prolonged periods of time (Asmussen and Kjeldsen 1975, Morrow *et al.* 1988, Rubinstein *et al.* 1991). Moreover, exposure of animals and endothelial cells to cigarette smoke was shown to increase the permeability to plasma proteins (Allen *et al.* 1988, Holden *et al.* 1989). In spite of these findings, the pathogenesis of smoking-induced vascular damage *in*

Fax +420 2 293 894
E-mail physres@biomed.cas.cz http://www.lf2.cuni.cz/physiolres/physiolres.htm

vivo has not yet satisfactorily been elucidated. Information about the effect of chronic passive smoking on the function of vascular smooth muscles, endothelial cells or nerve endings innervating the blood vessels is rather sparse (Li *et al.* 1994, McVeigh *et al.* 1996).

The purpose of this study was to examine the effect of prolonged passive smoking (lasting 3 weeks) in rabbits on a) plasma levels of catecholamines, b) neurogenic contraction of arteries induced by stimulation of sympathetic nerves, and c) endothelium-dependent relaxation of precontracted arteries induced by acetylcholine. This work has been presented as a preliminary communication (Török *et al.* 1998).

Methods

Experiments were performed on adult male Chinchilla rabbits (6 months old) which were fed with a standard pellet mixture. The rabbits were divided into two groups: control group and the group exposed to passive smoking. The model of passive smoking was as follows: the rabbits were placed in a child incubator and inhaled smoke from 3 cigarettes for 30 min twice daily over 21 days (smoking rabbits). The last smoking was performed 18 h before the *in vitro* experiments. Control animals were also placed in the incubator, but they inspired fresh air without cigarette smoke.

Rabbits were anesthetized with sodium thiopental (50 mg/kg i.v.) and killed by bleeding from the common carotid arteries. The heart was immediately removed and various anatomical parts of the heart were weighed. Isolated arterial rings were prepared according to the method described earlier (Török *et al.* 1993). The middle parts of the thoracic aorta and carotid arteries were rapidly removed, cleaned of excess fat and connective tissue and cut into rings about 4 mm in length. The rings were mounted on stainless steel hooks and suspended in an organ bath containing 20 ml modified Krebs solution which was bubbled with 95 % O_2/5 % CO_2 at 37 °C. One side of the tissue was connected by a thread to a force-displacement transducer (Sanborn FT 10) to measure changes in isometric contraction which were recorded with a polygraph,TZ 4200 (Labora).

The Krebs solution consisted of (in mmol/l): NaCl 118, KCl 5, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11, $CaNa_2$.EDTA 0.03. The resting tension of arterial rings was 20 mN. The preparations were allowed to equilibrate for 90 min before experimental observation. During this period, the Krebs solution was changed at 15-min intervals.

Endothelium-dependent relaxations of arteries were measured after an active tension had been elicited with phenylephrine (10^{-6} mol/l). When the contractile response had reached a plateau, acetylcholine was added to the organ bath in a cumulative manner. All preparations were pretreated with indomethacin (10^{-5} mol/l) to avoid the possible participation of prostaglandins in endothelium-dependent relaxation.

Neurogenic contractions were obtained by electrical stimulation of intramural nerves in the vessel wall. Using a Grass S88 stimulator, electrical stimulation was delivered to the tissue through a pair of platinum electrodes placed in parallel to the vessel ring. To obtain neurogenic contractile responses and to avoid direct stimulation of smooth muscles, the following parameters were used: square-wave pulses 0.5 . ms duration, supramaximal voltage, 1-32 Hz, duration of stimulation 20 s.

Plasma noradrenaline, adrenaline and dopamine levels were assayed radioenzymatically (Peuler and Johnson 1997).

Drugs

The following drugs were used: phenylephrine, acetylcholine chloride, indomethacin, sodium nitroprusside, tetrodotoxin (all from Sigma) and nicotine tartrate (Aldrich). Drugs were diluted in distilled water, except for indomethacin which was dissolved in 0.2 mol/l Na_2CO_3.

Statistical analysis

The results were expressed as means ± S.E.M. Differences between means were evaluated by one-way analysis of variance (ANOVA) or Student's t-test for unpaired observations. $P<0.05$ was taken as statistically significant.

Results

Cardiac mass indexes

As shown in Table 1, body weight did not differ between the control and smoking group of rabbits. Heart weight, right and left ventricular weight are expressed as HW/BW, RV/BW and LV/BW (g/kg). HW/BW was significantly higher in rabbits exposed to smoke (2.15±0.90 g/kg) than in control rabbits (1.72±0.13 g/kg, $P<0.05$). LV/BW was also significantly increased in the smoking group (2.99±0.12 g/kg) compared with the control group (2.48±0.07 g/kg, $P<0.01$). RV/BW ratio did not differ between the control and smoking groups.

Table 1. Effect of passive smoking on body and heart weight, and cardiac mass indexes

	n	BW (kg)	HW (g)	RV (g)	LV (g)	HW/BW (g/kg)	RV/BW (g/kg)	LV/BW (g/kg)
Control	7	3.23±0.66	5.95±0.14	1.15±0.05	2.48±0.07	1.72 ±0.13	0.36 ±0.02	0.77±0.02
Smoke	6	3.15±0.84	6.76±0.23*	1.25±0.06	2.99±0.12**	2.15±0.09*	0.40±0.02	0.95±0.05**

*Values are means ± S.E.M. BW - body weight, HW - heart weight, RV - right ventricle, LV - left ventricle. Significant differences from controls: * P<0.05, ** P<0.01.*

Plasma catecholamines

Plasma noradrenaline, adrenaline and dopamine concentrations in control and smoking rabbits are illustrated in Figure 1. The plasma noradrenaline level in control animals was 22.2±4.9 nmol/l; whereas it was elevated to 56.1±17.4 nmol/l in smoking rabbits (P<0.05). No significant increase in plasma adrenaline or dopamine concentration was associated with prolonged passive smoking.

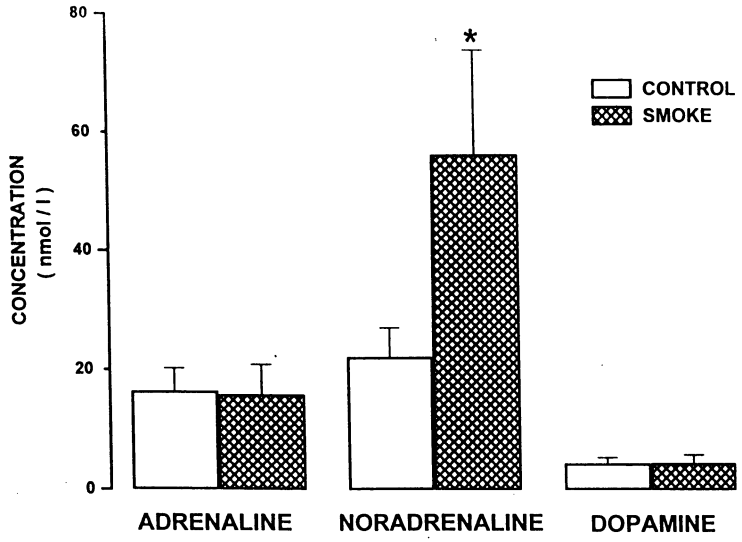

Fig. 1. *Effect of prolonged passive smoking on plasma adrenaline, noradrenaline and dopamine concentrations. Values are means ± S.E.M. of 6-7 experiments. *P<0.05 compared with corresponding control value.*

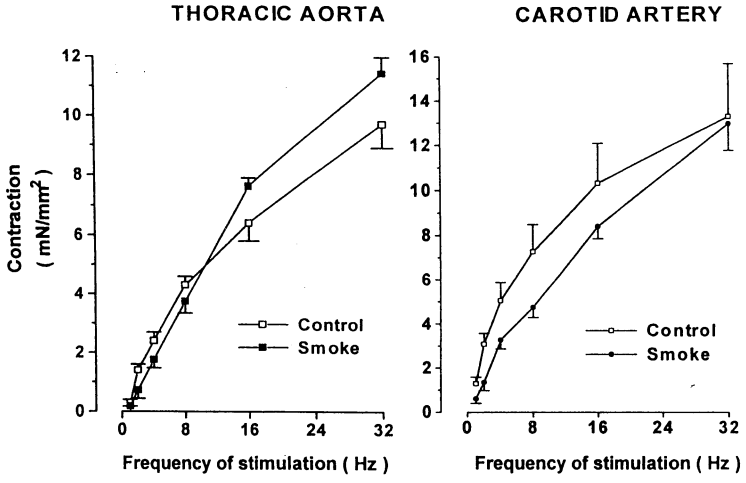

Fig. 2. *Effect of prolonged passive smoking on contractile responses to adrenergic nerve stimulation at various frequencies (1 – 32 Hz) in thoracic aorta and carotid artery. Values are means ± S.E.M. of 6-7 experiments.*

Effect of exogenous nicotine

Acute administration of nicotine (10^{-4} mol/l) into the incubation bath transiently enhanced neurogenic contraction of the carotid artery in control animals and this effect disappeared after 40-60 min (Fig. 3B). On the other hand, administration of nicotine had no effect on the magnitude of neurogenic contraction of the carotid artery from rabbits exposed to passive smoking for a period of three weeks (Fig. 3A).

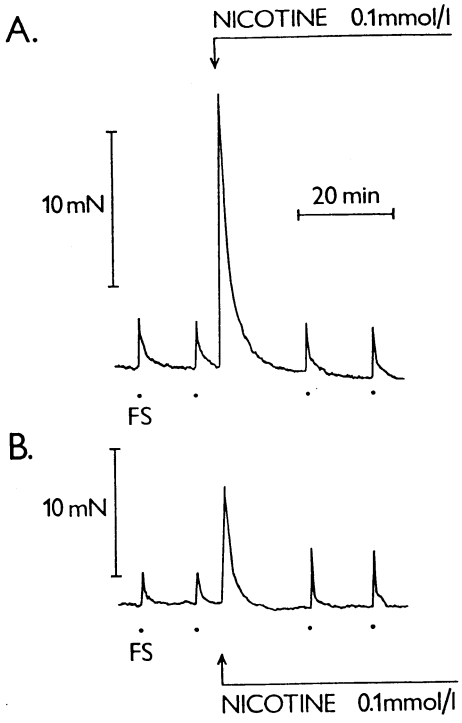

Fig. 3. *Representative recordings of the effect of nicotine (0.1 mmol/l) on neurogenic contractions of carotid artery induced by electrical field stimulation (FS) at 8 Hz from control rabbit **[B]** and from rabbit exposed to prolonged passive smoking **[A]**. Nicotine was added as indicated and kept in the bath for the rest of the experiment.*

Endothelium-dependent relaxation

The phenylephrine-precontracted thoracic aorta relaxed in response to increasing concentrations of acetylcholine; at 10^{-5} mol/l the relaxation averaged 89.7±6.5 % in the controls. In smoking rabbits, the relaxation was smaller and reached only 44.8±6.5 % at the maximal concentration of acetylcholine (Fig. 4).

Similar findings were observed in the carotid artery, where the maximal relaxation at 10^{-5}mol/l of acetylcholine was 92.7±4.8 % in controls, while after passive smoking it decreased to 43.3±4.2 % ($P<0.01$).

Sodium nitroprusside

There was no difference in the relaxation of thoracic aorta to nitroprusside ($3x10^{-7}$ mol/l) between control rabbits (98.6±2.5 %, n=7) and rabbits exposed to prolonged passive smoking (97.1±4.2 %, n=5).

Discussion

The present study showed that prolonged passive smoking in rabbits resulted in 1) the development of left ventricular hypertrophy, 2) a rise in plasma noradrenaline and 3) impairment of endothelium-dependent relaxation induced by acetylcholine in the carotid artery and thoracic aorta.

The deleterious effect of smoking on the cardiovascular system has been generally accepted (Benowitz and Gourlay 1997), but only a few papers have paid attention to the problem, whether passive smoking was associated with endothelial dysfunction in peripheral blood vessels (Rubinstein *et al.* 1991, Higman *et al.* 1993, Celermajer *et al.* 1996, Sumida *et al.* 1998).

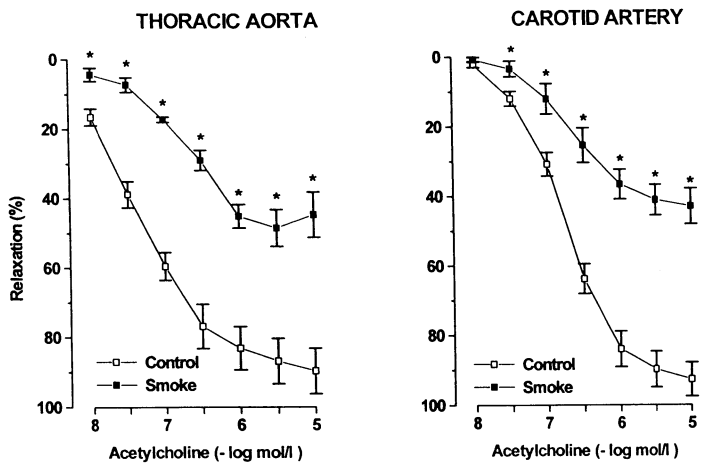

Fig. 4. *Endothelium-dependent relaxation of thoracic aorta and carotid artery induced by acetylcholine in control rabbits and in rabbits exposed to prolonged passive smoking. Arteries were precontracted by phenylephrine (10^{-6}mol/l). Values are means ± S.E.M. of 6-7 experiments. *$P<0.05$ compared with corresponding control value.*

Smoking may influence vascular functions *via* hemodynamic consequences of increased sympathetic neural stimulation and systemic catecholamine release (Winniford *et al.* 1986, Nakamura and Hayashida 1992, Benowitz and Gourlay 1997). It has previously been shown that cigarette smoke increased plasma levels of noradrenaline, the sympathetic neurotransmitter, as well as of adrenaline, the neuromodulating hormone (Cryer *et al.* 1976). Indeed, we did find a slight increase of plasma noradrenaline, which may have been due to enhanced sympathetic nerve activity and a local release of transmitter from adrenergic terminals within the cardiovascular system. Although we did not measure the blood pressure, it was reported earlier that smoke exposure caused an increase of blood pressure and an increase in sympathetic nerve activity with elevation of plasma noradrenaline (Peterson *et al.* 1983, Nakamura and Hayashida 1992, Houdi *et al.* 1995). In experimental animals, cigarette smoke may be considered a powerful stimulus. Similarly, the cardiovascular system of non-adapted nonsmokers exposed to passive smoke may be more sensitive than that of active smokers because of the lack of a fully developed protective response.

Long-lasting continuous infusion of noradrenaline in rats elevated systolic blood pressure and caused pronounced hypertrophy of the heart after 4 days of noradrenaline infusion (Johnson *et al.* 1983). The potential blood pressure increase may have been involved in the development of left ventricular hypertrophy observed in this particular model of passive smoking (Šimko *et al.* 1999). The increased plasma noradrenaline and possibly the increased blood pressure in smoking rabbits may have contributed to the development and maintenance of hypertrophy of the left ventricle in our experiments.

Cigarette smoke is known to contain a number of various substances, such as nicotine, carbon monoxide, tar, NO-derived free radicals and large amounts of oxygen free radicals, which may directly or indirectly contribute to the impairment of functional integrity of the endothelium. Nicotine, a major component of smoke, has often been considered a mediator of vascular injury. Nedergaard and Schrold (1977) showed that nicotine transiently enhanced neurogenic contractions of the rabbit isolated pulmonary artery by stimulation-induced transmitter release from adrenergic terminals. In our experiment, the application of exogenous nicotine to the incubation medium after short-term transient contractions also resulted in modest transient enhancement of neurogenic contractions of the carotid artery, but only in control rabbits which breathed clean air, whereas neurogenic contractions were not changed in the artery from smoking rabbits. This is in accord with the findings of Li and coworkers (Li and Duckles 1993, Li *et al.* 1994) showing that nicotine does not augment contractile responses to adrenergic nerve stimulation in the rat tail artery.

Impairment of acetylcholine-induced relaxation of the thoracic aorta and carotid artery from smoking rabbits may be the result of interactions of several factors:

a) The reduction of endothelium-dependent responses repeatedly found to occur not only in active smokers but also in individuals exposed to passive smoking appears to be caused by impairment of endothelial cells by cigarette smoke (Asmussen and Kjeldsen 1975, Morrow *et al.* 1988, Rubinstein *et al.* 1991, Kugiyama *et al.* 1996, Sumida *et al.* 1998). Cigarette smoke has been shown to contain a considerable amount of oxygen-derived free radicals, such as superoxide anions or hydrogen peroxide, which may inactivate NO continuously released from endothelial cells (Gryglewski *et al.* 1986, Rubányi and Vanhoutte 1986). The reduction of acetylcholine-induced relaxation may thus be mediated, at least in part, through the degradation of released EDRF (NO) by oxygen-derived free radicals present in smoke, as reported by Murohara *et al.* (1994) and Ota *et al.* (1997).

b) The attenuation of acetylcholine-induced relaxation after passive smoking may be related to inhibition of prostaglandin synthesis, since the cigarette smoke extract inhibits the synthesis of vasoactive prostaglandins (Jeremy *et al.* 1985). Our experiments with indomethacin present in the incubation medium, however, showed the relaxation of arteries in response to acetylcholine to be independent of the cyclooxygenase pathway. In addition, we found that prostaglandins did not participate in endothelium-dependent relaxations in the rabbit thoracic aorta (Török *et al.* 1993).

c) Nicotine was reported to produce selective impairment of endothelium-dependent vasodilatation (Mayhan and Patel 1997). The relaxation of aortic strips to acetylcholine was also significantly decreased in nicotine-treated rats (Hui *et al.* 1997). On the other hand, several studies failed to demonstrate significant functional alterations in vascular properties during exposure to nicotine (Jeremy *et al.* 1985, Allen *et al.* 1988, Mayers *et al.* 1988). In our experiments on the thoracic aorta from nonsmoking rabbits, acute addition of nicotine to the incubation medium was without significant effect. Nicotine does not seem to be the

decisive factor involved in the alteration of endothelium-dependent relaxation.

d) Elevated endothelin plasma levels have been suggested as the causal agent in the changed vascular response. However, the increase in plasma endothelin after cigarette smoking is a transitory phenomenon restricted to the first 10 min after the onset of smoking (Goerre *et al.* 1995). Thus it does not seem to be responsible for the reduction of endothelium-dependent relaxation.

In conclusion, the present study demonstrated that long-term passive smoking in rabbits impaired endothelium-dependent relaxation of systemic arteries. Furthermore, three weeks of passive smoking was associated with hypertrophy of the left ventricle. Additional studies are needed to assess whether the same functional changes are also observed in arterial resistance vasculature.

Acknowledgements

This study was supported by grant No. 1/4112/97 from the Ministry of Education Slovak Republic and Slovak Grant Agency for Sciences No. 2/4100/97.

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Reprint requests

Dr. J. Török, Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Sienkiewiczova 1 813 71 Bratislava, Slovak Republic. E-mail:torok@unpf.savba.sk