Vascular Mechanisms

Acute Effects of Passive Smoking on Peripheral Vascular Function

Jean-François Argacha, Dionysis Adamopoulos, Marko Gujic, David Fontaine, Nadia Amyai, Guy Berkenboom, Philippe van de Borne

Abstract—Environmental tobacco smoke (ETS) acutely affects peripheral and coronary vascular tone. Whether ETS exerts specific deleterious effects on aortic wave reflection through nicotine exposure, whether they persist after ETS cessation, and whether the smoke environment impairs microvascular function and increases asymmetrical dimethyl-arginine levels are not known. We tested these hypotheses in a randomized, crossover study design in 11 healthy male nonsmokers. The effects of 1 hour of exposure to ETS, as compared with a nontobacco smoke and normal air, on augmentation index corrected for heart rate and skin microvascular hyperemia to local heating were examined. Augmentation index increased both during (P=0.01) and after (P<0.01) the ETS session but remained unchanged in the nontobacco smoke session when compared with normal air. Nicotine levels after the exposure were related to the peak rise in augmentation index (r=0.84; P<0.01), denoting a predominant role of nicotine in ETS vascular effects. This was confirmed in a second set of experiments (n=14), where the sublingual administration of nicotine was associated with an acute impairment in wave reflection as compared with placebo (P=0.001). Both ETS and nontobacco smokes increased plasma asymmetrical dimethyl-arginine levels (P<0.001), but only ETS reduced the late rise in skin blood flow in response to heating (P=0.03). In conclusion, passive smoking specifically increases aortic wave reflection through a nicotine-dependent pathway and impairs microvascular function, even after the end of the exposure. However, both tobacco and nontobacco passive smoking inhalation increase plasma asymmetrical dimethyl-arginine levels.

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Key Words: passive smoking ■ nicotine ■ endothelium ■ wave reflection ■ nitric oxide

Exposure to environmental tobacco smoke (ETS) has been recognized recently as a strong contributor to cardiovascular mortality, accounting for >50 000 deaths annually in the United States.1 In recent years, extensive research has elucidated many aspects of the long-term ETS-related adverse effects on the cardiovascular system.2 However, the use of normal air inhalation as a control limits the interpretation of the physiopathological processes underlying the acute cardiovascular toxicity of ETS.3–4 Whether lung irritation and/or the stress provoked by smoke inhalation generate a nonspecific cardiovascular reaction5 and whether this can explain the effect of ETS2–4 are not known. We, therefore, decided to test the hypothesis that the vascular effects of ETS cannot simply be ascribed to a nonspecific reaction to smoke. This would provide further clear-cut evidence that ETS exerts specific deleterious cardiovascular effects beyond those of smoke pollution.

To further prove the specificity of the toxic effects of ETS, our second new hypothesis was that the deleterious vascular effects of ETS would be related to the rise in plasma nicotine levels. The above-mentioned studies2–4 did not determine plasma nicotine. The role of nicotine in the changes in aortic wave reflection to ETS2–4 can be suspected from the established sympathoexcitatory effects of nicotine in active smokers6 but has not been demonstrated previously to our knowledge.

Third, previous human studies on the acute effects of passive smoking have considered only the cardiovascular response during the acute smoke exposure.2,4 However, ETS exposure is an intermittent phenomenon, and it is unknown whether the acute deleterious effects of ETS persist after the smoke exposure. We tested the new hypothesis that the negative effects of ETS persist during periods where subjects again breathe normal air. This would reveal that the toxicity of ETS exposure is underestimated when only the duration of the acute exposure is taken into account.

Fourth, the effects of ETS and nontobacco smoke on microvascular function are unknown in humans. Because ETS exposure induces endothelial dysfunction of large conductance arteries,7 it was also assessed the effects of ETS on microvascular function by evaluating the skin hyperemic response to heating.
Finally, several in vitro studies have emphasized the adverse effects of chronic cigarette smoke exposure on endothelial NO bioavailability, particularly through a competitive inhibition of NO synthase by asymmetrical dimethylarginine (ADMA).6,9,10 Effects of nontobacco smoke on ADMA levels are unknown and were compared with those of ETS in our study. We believed that this was important to provide a broader understanding of the effects of smoke exposure on the endothelial arginine metabolism.

Materials and Methods

Subjects
Twenty-five healthy male subjects with normal physical examination who were on no medication were enrolled in the study. All were nonsmokers, and they were not regularly exposed to ETS. The ethical committee approved the study protocol, and informed written consent was obtained from each subject.

Study Design

Passive Smoking Protocol
During 3 sessions at 1-week intervals, 11 healthy male subjects (mean age: 24.6±3.3 years; body mass index: 22.5±2.1 kg/m²) were randomly exposed for 1 hour to sidestream tobacco smoke, sidestream nontobacco smoke, and normal air. We used a randomized, single-blinded, placebo-controlled, crossover study design with identical air particle densities during ETS and nontobacco smoke exposure. All of the measurements were performed in a quiet room with the subject resting, but not sleeping, in the supine position under carefully standardized conditions. All of the subjects abstained from meals, alcohol, and coffee beverages for 12 hours before each study period. Subjects were excluded from the experiment if they had used nonsteroidal antiinflammatory drugs for ≥3 days before each visit.

After a resting period of 20 minutes, subjects were exposed to the tested smoke or air for 1 hour. We used sidestream smoke from commercial cigarettes (Lucky-Strike; nicotine: 0.8 mg; tar: 10.0 mg) for tobacco smoke and herbal cigarettes (NTB, Arkopharma; nicotine: 5.0 µg; tar: 3.0 mg) for nontobacco smoke. Standardized smoke exposure conditions were achieved by placing a hermetic Plexiglas box of 0.1 m³ over the head of the subject. For the duration of the experiment, the box was ventilated by a motorized system composed of 2 separate tubes (input and output) ensuring a constant airflow through the box. Subjects breathed through a low-resistance mouthpiece with a nose clip to ensure exclusive mouth breathing. Expired gases were brought out of the box through a nonrebreathing valve. Eyes were protected by glasses that were delicately placed on the eyes to avoid any pressure to the ocular globes. A total of 6 cigarettes were lit in the input tube, 1 every 10 minutes for 1 hour, to allow a steady state of nicotine and tobacco smoke concentrations.

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Nicotine Protocol
To assess the role played by nicotine in aortic wave reflection changes produced by passive smoking, a separate group of 14 male healthy subjects (mean age: 24±4 years; body mass index: 24±0.5 kg/m²) were randomly exposed to a 2-mg nicotine sublingual tablet (Nicorette microtab, nicotine betadex, Pfizer) or to a placebo tablet, using a randomized, double-blind crossover study design. Similar experimental conditions than those of the passive smoke protocol were applied.

Measures

Aortic Wave Reflection Assessment
Aortic wave reflection was assessed noninvasively in all of the subjects at baseline, at 10-minute intervals during the 60 minutes of smoke exposure, and 20 minutes after smoke cessation, using a fully automated and validated, commercially available system (Sphygmo-
Table 1. Baseline Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Tobacco Smoke, Mean±SEM</th>
<th>Nontobacco Smoke, Mean±SEM</th>
<th>Normal Air, Mean±SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral SBP, mm Hg</td>
<td>107±2</td>
<td>104±4</td>
<td>104±4</td>
<td>NS</td>
</tr>
<tr>
<td>Peripheral DBP, mm Hg</td>
<td>61±1</td>
<td>57±3</td>
<td>58±2</td>
<td>NS</td>
</tr>
<tr>
<td>Mean BP, mm Hg</td>
<td>73±1</td>
<td>70±3</td>
<td>70±2</td>
<td>NS</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>56±1</td>
<td>57±2</td>
<td>57±2</td>
<td>NS</td>
</tr>
<tr>
<td>Central SBP, mm Hg</td>
<td>89±1</td>
<td>86±3</td>
<td>86±3</td>
<td>NS</td>
</tr>
<tr>
<td>Central DBP, mm Hg</td>
<td>61±1</td>
<td>57±3</td>
<td>58±2</td>
<td>NS</td>
</tr>
<tr>
<td>A1x, %</td>
<td>−14±2</td>
<td>−13±2</td>
<td>−13±1</td>
<td>NS</td>
</tr>
<tr>
<td>Transit time, ms</td>
<td>172±4</td>
<td>166±5</td>
<td>172±5</td>
<td>NS</td>
</tr>
<tr>
<td>Carboxyhemoglobin, %</td>
<td>1.3±0.1</td>
<td>1.3±0.1</td>
<td>1.5±0.1</td>
<td>NS</td>
</tr>
</tbody>
</table>

SBP indicates systolic BP; DBP, diastolic BP; A1x, A1x corrected for heart rate; NS, nonsignificant.

Statistical Analysis

Data are expressed as means±SEMs. Baseline hemodynamic parameters and carboxyhemoglobin levels in tobacco smoke, nontobacco smoke, and normal air sessions were compared using a 1-way ANOVA (SPSS Windows 13.0). A 2-way repeated-measures ANOVA was used to detect significant changes in variables over time between the experimental sessions and separately during and after the 3 exposures, after applying a Bonferroni correction for multiple comparisons. A 2-way repeated-measures ANOVA was also used to detect changes in skin blood flow during the heating provocation test performed before and after the 3 experimental sessions. Smoke particle concentrations and serum nicotine levels were compared using a Student’s paired t test. Carboxyhemoglobin and ADMA changes after the smoke exposure were compared using a 2-way repeated-measures ANOVA (time×smoke interaction). The relation among serum nicotine levels, A1x corrected for HR, and skin blood flow heating test changes was assessed using Pearson’s correlation coefficient. Significance was assumed at P<0.05.

Results

Baseline Characteristics

There were no differences in all of the baseline hemodynamic parameters or in carboxyhemoglobin levels among the 3 sessions (Table 1).

Smoke Exposure

All of the subjects were exposed equally to smoke during the tobacco and nontobacco smoke sessions, as assessed by smoke particle concentrations. The carboxyhemoglobin levels reached after 60 minutes of nontobacco smoke exposure were slightly higher than with tobacco smoke (P<0.05; Table 2).

Plasmatic Nicotine and ADMA Levels

Nicotine levels after 60 minutes of tobacco smoke exposure were 10-fold higher compared with the level reached after nontobacco smoke exposure (P<0.001; Table 2). Both tobacco and nontobacco smokes increased plasmatic ADMA levels (Figure 1), respectively, from 0.67±0.01 to 1.12±0.1 μmol/L and from 0.68±0.01 to 1.16±0.1 μmol/L (time effect: P<0.001; time×smoke interaction: P value not significant).

Table 2. Effects of Smoke Exposure on Nicotine, Carboxyhemoglobin, and Particle Matter

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Tobacco Smoke, Mean±SEM</th>
<th>Nontobacco Smoke, Mean±SEM</th>
<th>Normal Air, Mean±SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM 1.0, μg/m³</td>
<td>52±2</td>
<td>51±2</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>PM 2.5, μg/m³</td>
<td>300±19</td>
<td>270±32</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>PM 7.5, μg/m³</td>
<td>400±40</td>
<td>320±40</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>PM 10.0, μg/m³</td>
<td>400±40</td>
<td>320±40</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Carboxyhemoglobin, %</td>
<td>2.1±0.1</td>
<td>2.5±0.1</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Serum nicotine levels, ng/mL</td>
<td>3.1±0.4</td>
<td>0.3±0.1</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

NS indicates nonsignificant.

Heart Rate

HR increased in the tobacco smoke session (P<0.04), reaching a peak level 50 minutes after exposure initiation. Moreover, HR values returned to baseline after the end of the smoke exposure. In the nontobacco session, no effects on HR were noted during or after smoke exposure. However, the interaction between the 2 sessions (tobacco versus nontobacco smoke) remained nonsignificant during and after the exposure (Figure S1, please see http://hyper.ahajournals.org).

Blood Pressure

Tobacco smoke had no effect on peripheral or aortic BP during or after the smoke exposure; similar observations were made during and after the nontobacco smoke exposure (Figure S1). The interaction between the 2 sessions (tobacco versus nontobacco smoke) remained nonsignificant for systolic and diastolic BP at the periphery and at the aorta.

Aortic Wave Reflection

A1x corrected for HR increased both during (P=0.01) and after (P<0.01) the tobacco smoke session but remained unchanged in the nontobacco smoke session. The interaction between the 2 sessions (tobacco versus nontobacco smoke)
was significant for both exposure ($P = 0.04$) and postexposure ($P = 0.05$) periods (Figure 2).

The largest increases in serum nicotine levels after the exposure to tobacco smoke were related to the greatest increase in AIx ($r = 0.84; P = 0.01$) observed 50 minutes after the initiation of ETS exposure (Figure S2). In the additional study, nicotine sublingual administration was associated with an increase in AIx corrected for HR ($P = 0.001$) throughout the study (Figure S3).

Transit Time
Transit time decreased both during ($P = 0.02$) and after ($P < 0.01$) the tobacco smoke session, but remained unchanged in the nontobacco smoke session. The interaction between the tobacco and nontobacco smoke sessions was significant for both exposure ($P = 0.02$) and postexposure ($P = 0.01$) periods (Figure 2).

Heated Skin Blood Flow Reactivity
None of the interventions affected skin blood flow recorded in baseline conditions before the initiation of the heating protocol. Normal air and nontobacco smoke did not affect the early and late skin blood flow responses to heating (Figure 3). In contrast, the skin blood flow response to heating decreased after the tobacco smoke exposure ($P = 0.02$) compared with the baseline response. This was because of a decrease in the late phase of the reaction after the smoke exposure ($P = 0.03$), whereas the early phase of the reaction remained unchanged (Figure 3).

**Discussion**

The main new findings of our study are as follows: (1) ETS exposure induces an acute increase in central wave reflection...
and a decrease in skin microvascular dilatation, when compared with nontobacco smoke; (2) increased central wave reflection is linked to the levels of nicotine achieved with ETS; (3) these modifications persist ≤20 minutes after ETS cessation; and (4) smoke inhalation, regardless to the tobacco content, is followed by a plasmatic increase in ADMA. Hence, large and small arterial vascular functions are altered by acute exposure to tobacco smoke. Hemodynamic changes provoked by ETS are the result of a primary nicotinic toxic mechanism acting on the vascular tree and are not mediated by a sensory stimulation or a stressful reaction to the smoke itself. In addition, our study suggests that the toxicity of ETS is markedly underestimated when only the direct exposure time is taken into account.

ETS Effects on Aortic Wave Reflection: The Role of Nicotine

In a previous study, 1 hour of ETS exposure acutely increased AIx by 16%. However, this reaction could be interpreted as the sum of 3 different effects: a placebo effect, a cardiovascular response secondary to sensory stimulation and stress by smoke, and a specific effect of the tobacco smoke on vascular function. In contrast, our results demonstrate that ETS exposure produces a marked change in the aortic waveform through a primary toxicity induced by tobacco on the vascular tree.

Enhanced arterial wave reflection, because of a change in peripheral vascular reflection site, and/or augmented pulse wave velocity can generate an increase in AIx. Although pulse wave velocity was not measured directly, the aortic pulse transit time, which has been used as a surrogate marker of pulse wave velocity, was decreased by tobacco smoke. This finding suggests that the intensified arterial wave reflection in the aorta could be explained by a reduction in vessel compliance after ETS exposure. In patients with coronary artery disease, invasive measurements of the aortic pressure-diameter loop have demonstrated that 4 minutes of ETS exposure were able to decrease aortic distensibility. Our results are in accordance with this finding and suggest that changes in the aortic waveform in response to passive smoke are favored by active stiffening of the aorta. However, concomitant changes in aortic wave reflection resulting from an increased peripheral vascular tone, as reported previously in active smokers, may also have played a role. Previously, short-term ETS exposure (15 minutes) did not interfere with forearm vascular resistance measured by plethysmography. However, this segmental analysis of vascular resistance cannot rule out a systemic arteriolar vasconstriction after a more prolonged ETS exposure and larger increases in plasma nicotine. Further studies are required to characterize the possible differential effects of impaired arterial compliance and early reflection site in the rise in AIx with ETS.

The comparison of the vascular response to passive smoking of a real cigarette and an herbal cigarette reveals a more toxic effect of the tobacco smoke. At first glance, the main toxic difference between the 2 smokes may reside in the nicotine content of tobacco smoke. Indeed, it is widely accepted that nicotine has pleiotropic actions that could affect vasomotor regulation. Our results reveal a significant relationship between the changes in aortic waveform and the nicotine levels reached in plasma after 1 hour of tobacco smoke exposure (Figure S2). This relationship was confirmed in our second protocol demonstrating an acute impairment in arterial wave reflection produced by a pure nicotinic stimulus. This finding provides novel information regarding the role played by nicotine in the smoke-related vascular damage. However, the exact mechanisms responsible for the nicotine-induced rise in arterial wave reflection, the effect of the aortic hemodynamic changes on myocardial perfusion, and the possibility of tolerance development after chronic exposure to nicotine require further investigation.

Effects of ADMA

An important contribution of our study also resides in the demonstration that nontobacco smoke increases plasmatic ADMA levels, a competitive inhibitor of NO synthase. This occurred to a similar extent as with tobacco smoke. Usually the most common mechanism leading to accumulation of ADMA involves impaired metabolism by dimethylarginine dimethylaminohydrolase, an enzyme extremely sensitive to oxidative stress. Thus, we hypothesized that the free radical exposure, arising from identical particulate exposure of both smokes, produces the same inhibition of dimethylarginine dimethylaminohydrolase activity and generates the same degree of competitive inhibition of NO synthase. We believe that these results further add to the escalating evidence that exposure to polluted air is associated with short-term adverse cardiovascular effects. Increased ADMA levels in our study, not reported previously in vivo, as well as with nontobacco smoke, provide a further indication on how air pollution may elicit cardiovascular events.

Tobacco-Free Cigarette: A Safe Smoke?

Contrary to tobacco smoke, nontobacco smoke exposure produced no significant change in arterial waveform and skin blood flow, suggesting a key role of the nicotine content of smoke, at least in acute conditions. Despite this “relative” safety, herbal cigarette smoke increased plasmatic ADMA levels and exposed the subject to the same level of particle matter as ETS. Even if a previous study did not identify acute sympathetic or peripheral vascular effects of carbon monoxide, carboxyhemoglobin increase from nontobacco smoke exposure has some detrimental effect on myocardial blood flow. For these reasons, we believe that herbal smoke cannot be considered as a safe cardiovascular smoke, especially because the role of fine particle matter in the endothelial toxicity of cigarette smoke and air pollutants remains unclear.

Perspectives: Microvascular Effect of ETS

ETS exposure has immediate effects on endothelium-dependent vasodilatation of large arteries, but our study also reveals an impairment of vascular reactivity in small-size vessels. Many studies have established the physiological substrate of skin thermal hyperemia. Local heating evoked an initial vasodilatation followed by a plateau, which is mediated by NO. However, even if the role of endothelium-derived NO is predominant, endothelium-
dependent hyperpolarizing factors are also involved in this reaction.28 Consequently, the microvascular impairment occurring after 1 hour of ETS exposure may be interpreted as a decrease in endothelium-dependent hyperpolarizing factor and/or endothelium-derived NO bioavailability. This will require further studies to better identify the mechanisms involved in the microvascular endothelial impairment observed with ETS. Moreover, only tobacco smoke impaired microvascular function in the presence of an increase in plasmatic ADMA. This paradox could be explained by a specific ability of tobacco smoke to decrease endothelial production of endothelium-dependent hyperpolarizing factor and/or NO, because nicotine can directly impair NO synthase expression,6 whereas acrolein can increase endogenous reactive oxygen species produced by NADPH activation.29 This also requires additional experiments, which will further underscore the importance of achieving a complete smoke-free environment.30

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Disclosures
None.

References

Argacha et al Acute Vascular Effects of Passive Smoking 1511
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Running title: Acute vascular effects of passive smoking

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Figure S1: Changes in peripheral blood pressure and heart rate during and after the 3 experimental sessions. Values expressed as mean ± SEM. Abbreviations as in table 1.
**Figure S2:** Correlation analysis between plasma nicotine levels and the rise in Augmentation Index corrected for heart rate (n=10).
**Figure S3:** Changes in Augmentation Index corrected for heart rate after the sublingual administration of a 2 mg nicotine and a placebo tablet (n=14).