Clinical Investigation and Reports

Diminished Vascular Response to Inhibition of Endothelium-Derived Nitric Oxide and Enhanced Vasoconstriction to Exogenously Administered Endothelin-1 in Clinically Healthy Smokers

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Background Smoking is a major risk factor for the development of atherosclerosis. Because endothelial dysfunction may be a marker for future atherosclerosis, we investigated the effects of smoking on endothelium-dependent control of vascular tone. Methods and Results The effects of brachial arterial infusions of Nω-monomethyl-L-arginine (L-NMMA), a nitric oxide synthesis inhibitor; sodium nitroprusside; endothelin-1; and norepinephrine on forearm blood flow (strain-gauge plethysmography) were compared in 29 long-term smokers and 16 nonsmokers. The acute effects of smoking on systemic hemodynamics, plasma catecholamines, and forearm vascular responses to these compounds were investigated in smokers only. Smokers did not differ from nonsmokers (n=16) regarding the vascular effects of sodium nitroprusside (n=13) or vasoconstriction due to norepinephrine and endothelin-1 (n=16). Low-dose endothelin-1-induced vasodilation, believed to reflect endothelial prostacyclin or nitric oxide release, was absent in smokers (n=16), and their increase of forearm vascular resistance (FVR) after L-NMMA (n=13) was impaired (35.6±27.9% versus 118.8±43.2%, P<.001). Short-term smoking (n=11) increased blood pressure, heart rate, and plasma epinephrine concentrations (P<.05 or less); enhanced endothelin-1–induced vasoconstriction (ΔFVR, 457±192% versus 254±143%, P<.01); and decreased norepinephrine–induced vasoconstriction (P<.05), but had no effect on the other interventions. Conclusions Long-term smoking is associated with a diminished nitric oxide–dependent component of basal vascular tone; and an impaired endothelium-dependent vasodilator response to low-dose endothelin-1 and short-term smoking enhances endothelin-1–induced vasoconstriction. Impaired endothelial control of vascular tone might reflect impairment of normal antiatherosclerotic endothelial functions in smokers, but the relevance of smoking-induced enhancement of endothelin-1 vasoconstriction remains to be determined. (Circulation. 1994;90:27-34.)

Key Words • smoking • endothelium • nitric oxide • flow • blood pressure

The vascular endothelium produces and releases vasoactive substances that appear to serve as important modulators of vascular tone. In addition, the endothelium is believed to play a key role in the maintenance of a normal vascular structure and the prevention of atherosclerotic changes. Although the original view of the response-to-injury theory of atherogenesis was centered on the morphological integrity of the endothelium, it is now believed that the loss of normal endothelial function rather than endothelial denudation is pivotal in the development of early atherosclerotic changes. In line with this view is evidence in animals that alterations of endothelial function and morphological changes characteristic of atherosclerosis coincide. Accordingly, changes in the endothelial control of vascular tone may be markers of early or future atherosclerotic vascular changes in humans, a view that is supported by observations that the presence of risk factors for the development of atherosclerosis is associated with endothelial dysfunction. Thus, the vasodilator response to acetylcholine, which is mediated through endothelial release of endothelium-derived relaxing factor and has been linked to endothelial synthesis and release of nitric oxide, is impaired in the presence of hypercholesterolemia in human coronary as well as forearm resistance vessels in vivo. Arterial hypertension is also associated with a diminished vasodilator response to acetylcholine and an abnormality of basal nitric oxide–mediated dilation in forearm resistance vessels. An impaired endothelial function has also been suggested in patients with diabetes mellitus. Finally, in addition to a disturbance of endothelial vasodilator and antiplatelet mechanisms, the endothelial vasoconstrictor peptide endothelin-1 may be involved in the pathogenesis of atherosclerosis.

Cigarette smoking is another important risk factor for the development of atherosclerosis, and its effects on endothelial prostacyclin production and the vessel wall–platelet interaction have received considerable in-
terest. Less information is available regarding the effects of smoking on other endothelial control mechanisms of vascular tone, but recent studies have described impaired acetylcholine-mediated coronary and flow-mediated forearm vasodilation in clinically healthy smokers. Because the study of endothelial control mechanisms of vascular tone may provide clues to the mechanisms leading to the development of atherosclerosis, we studied the effects of short- and long-term smoking on basal, nitric oxide–mediated vascular tone and on the vascular response to endothelin-1.

Methods

The study population was composed of 29 healthy male long-term smokers who had smoked at least one packet of cigarettes per day for 10 years (10 to 56 years; mean, 26 packets per day·years) and 16 healthy, age-matched male nonsmokers who had never smoked. All subjects had refrained from ingesting caffeine-containing beverages, and long-term smokers had abstained from smoking cigarettes for at least 8 hours before the start of the investigations. The study protocol was approved by the hospital ethical committee on the use of human subjects in clinical investigations, and written informed consent was obtained from all participants.

Forearm Blood Flow Measurements

Forearm blood flow was measured bilaterally by venous occlusion plethysmography. In short, a mercury-in-Silastic strain gauge was placed at the upper third of the forearm, which rested comfortably on a support slightly above the level of the heart. The strain gauge was coupled to an electronically calibrated plethysmograph (EC4, Hokanson). Venous occlusion was achieved by a blood pressure cuff applied proximal to the elbow and inflated to 40 mm Hg with a rapid cuff inflator (EC10, Hokanson). The hand was excluded from the circulation by inflating a pediatric blood pressure cuff placed around the wrist to 50 mm Hg above systolic pressure 1 minute before and during the forearm blood flow measurement to eliminate the unpredictable influence of arteriovenous shunts in the hand. Experiments were done on the left (experimental) forearm, and blood flow measurements on the right (control) arm served as a continuous control. Plethysiographic recordings were analyzed using a digitizing board and a suitably programmed computer. The mean value of four to six recordings obtained within 1 minute was taken for statistical evaluation. Heart rate was derived from the ECG. Forearm vascular resistance was calculated by dividing mean arterial pressure, obtained immediately after flow measurements, by forearm blood flow.

Preparations of Solutions for Infusions

All solutions were freshly prepared before each study. Endothelin-1 (Peptides International) and Nω-monomethyl-L-arginine (L-NMMA; Calbiochem AG) were diluted in Physiogel (gelatin solution 4%; MW, 22,000; Swiss Red Cross) to avoid binding to syringes or tubing. Infusions of Physiogel at volumes of up to 6 mL/min did not influence forearm blood flow to a measurable degree. Sodium nitroprusside was dissolved in 5% dextrose and protected from light. Norepinephrine (Dr G. Biichsel AG) and L-arginine (Hausmann AG) were dissolved in 0.9% saline. Intra-arterial infusions were performed with volumes between 0.15 and 0.8 mL/min for norepinephrine and sodium nitroprusside, respectively. For endothelin-1 and L-NMMA, volumes were hold constant at 2 mL/min and 1 mL/min, respectively, using a constant-speed infusion pump (Sage Instruments Inc).

Study Protocols

The studies started at 8:00 AM and lasted for approximately 5 to 6 hours. The subjects were supine in a quiet, air-conditioned room with a constant temperature (20° to 22°C). Forearm volume was measured by water displacement using the Archimedes principle, and drug doses were adjusted accordingly. With the patients under local anesthesia (lidocaine 2%), an 18-gauge catheter (Abbocath-T, Abbott) was inserted into the left brachial artery for regional drug infusion and recording of arterial pressure using a Statham P23 Pb pressure transducer. The subjects were allowed to rest for 30 minutes after completion of instrument preparation before measurement of basal forearm blood flow, intra-arterial blood pressure, and heart rate and arterial blood sampling for determination of plasma catecholamines and plasma cholesterol concentrations.

Studies in Smokers

Because of the long duration of endothelin-1–induced vasoconstriction in the human forearm, it was considered impossible to assess all aspects of the study in only one group of smokers. Accordingly, the effects of short-term smoking on endothelin-1–induced vasoconstriction and on nitric oxide–mediated control of vascular tone were investigated in two groups.

Smoking and Endothelin-1–Induced Vasconstriction

After measurement of baseline blood pressure, heart rate, and forearm blood flow, norepinephrine was infused in 11 smokers in three dosages (7.5, 20, and 40 ng·min⁻¹·100 mL⁻¹ forearm tissue) for 5 minutes each and forearm blood flow was recorded during the fifth minute of infusion. Approximately 20 minutes afterward, forearm blood flow had returned to basal values. Subsequently, endothelin-1 was infused in five increasing dosages (0.25, 0.5, 1.0, 25, and 50 ng·min⁻¹·100 mL⁻¹ forearm tissue) for 5 minutes, and measurements of forearm blood flow were obtained during the last minute. After the dose-response curve to endothelin was completed, forearm blood flow was allowed to return to baseline values, which took approximately 90 minutes. Then baseline hemodynamic recordings were obtained, and the subjects asked to smoke two cigarettes within 15 minutes. Five minutes later, blood pressure, heart rate, and forearm blood flow were recorded, and arterial blood was collected for determination of plasma catecholamines. Next, norepinephrine infusions and hemodynamic measurements were repeated, and the subjects asked to smoke one additional cigarette in the middle of the 20-minute waiting period. Finally, endothelin-1 infusions and the respective measurements were repeated in an identical fashion.

To test for a possible nonspecific effect unrelated to short-term smoking but rather to the long duration of the study, an identical timed drug infusion protocol was performed in 5 additional smokers who did not smoke before repeat infusions of norepinephrine and endothelin-1.

Smoking and Nitric Oxide–Mediated Control of Vascular Tone

After measurement of baseline blood pressure, heart rate, and forearm blood flow, we infused sodium nitroprusside (0.6 μg·min⁻¹·100 mL⁻¹ forearm tissue) in 8 smokers for 2 minutes, and measurements were repeated in the last minute of the infusion. After forearm blood flow had returned to basal values, L-NMMA was infused in three dosages (50, 100, and 200 μg·min⁻¹·100 mL⁻¹ forearm tissue) for 5 minutes each to inhibit nitric oxide synthesis, and hemodynamics were recorded during the fifth minute of each infusion. The dose of L-NMMA was based on previous studies in humans in which a brachial artery infusion of 100 μg·min⁻¹·100 mL⁻¹ L-NMMA reduced the vasodilator response to acetylcholine by approximately 70%. Thereafter, L-arginine was infused at a dosage of 850 μg·min⁻¹·100 mL⁻¹ forearm tissue for 7 minutes to reverse the inhibitory effect of L-NMMA on nitric oxide production. After approximately 40 minutes, forearm blood flow had returned to basal values. The subjects were
then asked to smoke two cigarettes in 15 minutes, and blood pressure, heart rate, and forearm blood flow were recorded and arterial blood was collected for determination of plasma catecholamines 5 minutes later. Next, the sodium nitroprusside infusion and the hemodynamic measurements were repeated, and the subjects were asked to smoke one additional cigarette during the following 20-minute waiting period. Finally, L-NMMA infusions and the respective measurements were repeated in an identical way.

In an additional 5 smokers, identically timed drug infusions were performed without smoking before repeat infusions of sodium nitroprusside and L-NMMA.

Studies in Nonsmokers

Because the short-term effects of smoking were not assessed in the 16 nonsmoking subjects, all investigations could be performed during the same study. Two different sequences of drug administration were used. In 11 subjects, baseline measurements were obtained followed by withdrawal of blood for determination of baseline plasma catecholamine and cholesterol concentrations. Then sodium nitroprusside, L-NMMA, L-arginine, norepinephrine, and endothelin-1 were infused allowing appropriate time between infusions for forearm blood flow measurements to return to baseline. In the remaining 5 subjects, the order was changed to L-NMMA first, followed by L-arginine, sodium nitroprusside, norepinephrine, and endothelin-1. However, changing the order of administration of sodium nitroprusside and L-NMMA did not affect the changes of forearm blood flow for either compound. Accordingly, the results of all 16 nonsmokers were pooled for analysis.

Statistical Analysis

Results are expressed as mean±SD. ANOVA with repeated measurements was used to evaluate norepinephrine, endothelin-1, and L-NMMA effects on the forearm vasculature. The influence of smoking status and the effects of short-term smoking on the overall effects of the interventions were assessed by two-way ANOVA with repeated measurements. The t test for paired observations was used to compare results obtained after a particular intervention with the corresponding control values, and the unpaired t test was used for comparison of smokers and nonsmokers. A two-tailed P value of <.05 was considered to indicate a significant difference. All calculations were performed using the STATVIEW II (Abacus Inc) statistical program.

Results

Long-term smokers and nonsmokers were well matched for age and did not differ with respect to plasma cholesterol, norepinephrine or epinephrine concentrations, heart rate, or diastolic blood pressure. Systolic blood pressure was lower in smokers, but this difference did not reach statistical significance (P<.06) (Table 1).

Long-term Smoking and Endothelial Control of Vascular Tone

Smokers and nonsmokers had similar baseline forearm flow and resistance values (Table 1). All pharmacological interventions were subjectively and objectively well tolerated, and none resulted in significant changes in blood pressure or heart rate, indicating a lack of systemic effect of the doses used. Thus, changes in forearm blood flow after a particular intervention directly reflected changes in vascular resistance attributable to its respective interference with the control of resistance vessel tone.

Brachial artery infusion of sodium nitroprusside increased forearm blood flow similarly in smokers (n=13) from 2.55±0.50 to 11.90±5.49 mL·min⁻¹·100 mL⁻¹ and in nonsmokers (n=16) from 2.49±0.59 to 12.2±4.45 mL·min⁻¹·100 mL⁻¹, respectively. The vascular responses to norepinephrine and endothelin-1 are summarized in Fig 1. Norepinephrine caused comparable reductions in forearm blood flow in smokers and nonsmokers. Likewise, the higher doses of endothelin-1 resulted in equal degrees of vasoconstriction. However, although the smallest dose of endothelin-1 resulted in an increase in forearm blood flow (2.62±0.78 versus 3.20±0.68 mL·min⁻¹·100 mL⁻¹, P<.01) in nonsmokers, it remained unchanged in smokers (2.70±0.60 versus 2.65±0.64 mL·min⁻¹·100 mL⁻¹). When comparing the vasoconstrictor potency of the two substances on a molar basis, endothelin-1 was approximately 15 times more potent than norepinephrine.

Blockade of nitric oxide synthesis by L-NMMA had a significantly (P<.01) greater effect on nonsmokers than in smokers (Fig 2). Thus, forearm blood flow in response to the highest L-NMMA dose decreased from 2.62±0.78 to 1.20±0.25 mL·min⁻¹·100 mL⁻¹ (−54.2±13.2%) in nonsmokers and from 2.65±0.66 to 2.11±0.72 mL·min⁻¹·100 mL⁻¹ (−25.7±21.5%) in smokers (P<.01). Accordingly, forearm vascular resistance increased by a maximum of 118.8±43.2% in nonsmokers and 35.6±27.2% in smokers (P<.001, effect of smoking status in two-way ANOVA).

Table 1. Baseline Characteristics of Long-Term Smokers and Nonsmokers

<table>
<thead>
<tr>
<th></th>
<th>Smokers (n=29)</th>
<th>Nonsmokers (n=16)</th>
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<tbody>
<tr>
<td>Age, y (range)</td>
<td>34.6±5.9 (24 to 53)</td>
<td>37.4±7.2 (22 to 56)</td>
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<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>108.8±8.3</td>
<td>116.0±8.4</td>
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<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>57.2±5.6</td>
<td>56.0±9.4</td>
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<tr>
<td>Heart rate, beats per minute</td>
<td>56.7±6.8</td>
<td>56.5±8.2</td>
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<tr>
<td>Plasma total cholesterol, mmol/L</td>
<td>4.8±1.0</td>
<td>4.7±1.0</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.02±0.14</td>
<td>1.15±0.22</td>
</tr>
<tr>
<td>Plasma norepinephrine, ng/L</td>
<td>178±61</td>
<td>166±53</td>
</tr>
<tr>
<td>Plasma epinephrine, ng/L</td>
<td>48.4±17.7</td>
<td>44.3±23.1</td>
</tr>
<tr>
<td>Forearm blood flow, mL·min⁻¹·100 mL⁻¹ tissue</td>
<td>2.57±0.61</td>
<td>2.49±0.59</td>
</tr>
<tr>
<td>Forearm vascular resistance, U</td>
<td>29.3±8.3</td>
<td>30.4±8.9</td>
</tr>
</tbody>
</table>

HDL indicates high-density lipoprotein. Values are given as mean±SD.
Short-Term Effects of Smoking

Potential time effects related to the study design were investigated in 10 smokers. Waiting without smoking before repeat drug infusions did not affect blood pressure (107.4±12.3/57.1±7.9 versus 106.5±12.4/56.3±8.4 mm Hg) or heart rate (56.4±6.8 versus 57.7±8.3 beats per minute) in these subjects. Likewise, vasoconstrictor responses to norepinephrine or endothelin-1 (n=5) or vascular responses to sodium nitroprusside and L-NMMA (n=5) were unchanged by waiting alone. The maximal responses to the respective compounds obtained during the baseline period and the repeat study (after waiting) are listed in Table 2.

Short-term smoking (n=19) caused sizeable increases in systolic blood pressure (from 106.8±10.3 to 115.9±10.4 mm Hg, P<.001) and heart rate (from 57.7±7.1 to 82.6±8.9 beats per minute, P<.001) and a small increase in diastolic pressure (from 55.2±6.7 to 57.7±8.1 mm Hg, P<.05). These changes were accompanied by a significant increase in plasma epinephrine (38.5±15.7 versus 52.1±23.5 ng/L, P<.05) but not norepinephrine (195.7±64.9 versus 196.5±75.1 ng/L) concentrations. Because blood pressure increased after smoking, changes in forearm vascular resistance rather than in forearm blood flow are presented for this part of the investigation.

Short-term smoking led to a slight but nonsignificant increase in forearm blood flow (2.15±.78 versus 2.42±1.05 mL·min⁻¹·100 mL⁻¹) that together with the increase in arterial pressure resulted in unchanged forearm vascular resistance (39.2±13.0 versus 38.0±17.5 U, n=19). No significant changes were induced by smoking with respect to the vascular effects of sodium nitroprusside or L-NMMA (n=8). Thus, forearm vascular resistance in response to sodium nitroprusside decreased by 74.7±12.7% (to 7.7±4.0 U) before and by 82.3±5.1% (to 6.7±4.1 U) after smoking. Forearm vascular resistance in response to the highest dose of L-NMMA (200 μg·min⁻¹·100 mL⁻¹) increased by 28.6±24.9% (to 43.5±21.8 U) before and by 40.2±40.2% (to 44.8±20.8 U) after smoking.

The influence of smoking on the vascular responses to norepinephrine and endothelin-1 was investigated in 11 smokers and is depicted in Fig 3. Although smoking significantly attenuated the increase in forearm vascular resistance to the highest dose of norepinephrine, the response to endothelin-1 showed an opposite pattern with an overall enhanced vasoconstrictor response (P<.05, effect of short-term smoking in two-way ANOVA with repeated measurements). This effect was

<table>
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<tr>
<th>TABLE 2. Effects of Waiting on Forearm Blood Flow Responses to Vasoconstrictor and Vasodilator Substances in Smokers</th>
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<tr>
<td>Changes of Forearm Blood Flow</td>
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<tr>
<td>Baseline study</td>
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<tr>
<td>Norepinephrine, 40 μg·min⁻¹·100 mL⁻¹ (n=5)</td>
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<tr>
<td>Endothelin-1, 50 ng·min⁻¹·100 mL⁻¹ (n=5)</td>
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<tr>
<td>Sodium nitroprusside, 0.6 μg·min⁻¹·100 mL⁻¹ (n=5)</td>
</tr>
<tr>
<td>L-NMMA, 200 μg·min⁻¹·100 mL⁻¹ (n=5)</td>
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</table>

L-NMMA indicates N°-monomethyl-L-arginine. Values are given as mean±SD. Repeat study refers to values obtained after waiting the same amount of time allowed for the other smokers to smoke a total of three cigarettes.
particularly pronounced for the two highest doses. In addition, the smallest dose of endothelin-1 that did not change forearm vascular resistance before smoking (30.4±10.5 versus 29.2±7.7 U) resulted in a significant increase after smoking (29.8±12.1 versus 33.4±11.0 U, P<.01).

Discussion

Despite the overwhelming epidemiological data linking smoking and atherosclerosis,17-19 little is known about the underlying mechanisms. The present study provides evidence that long-term cigarette smoking is associated with abnormalities of endothelial vasodilator control of resistance vessels in humans and an enhancement of the response to the vasoconstrictor peptide endothelin-1. These abnormalities may have far-reaching consequences by disturbing the normal interactions among the vessel wall, platelets, neutrophils, and macrophages, factors believed to be of importance in atherogenesis.3,4 These findings therefore may contribute to our understanding of the link between the long-term use of cigarettes and the adverse cardiovascular effects of smoking.

Impaired Endothelial Vasodilator Function in Long-Term Smokers

The present study suggests that the control of basal vascular tone mediated by the endothelium and the endothelial release of prostacyclin and/or endothelium-derived relaxing factor are impaired in long-term smokers. Nitric oxide, which accounts for most of the biological activity of endothelium-derived relaxing factor,7 is metabolized from l-arginine to citrulline.31 The arginine analogue L-NMMA stereospecifically inhibits the formation of nitric oxide in cell culture and vascular tissue,29 and a role of endothelium-derived relaxing factor in the maintenance of vascular tone in humans is demonstrated by a decrease in forearm blood flow after brachial arterial infusions of L-NMMA.29,32 This finding was confirmed in our nonsmoking subjects, whereas the response to L-NMMA in smokers was markedly impaired. Thus, our results suggest a reduction in basal nitric oxide–mediated vasodilation in long-term smokers who had no clinical evidence of vascular disease. We cannot determine from our data whether this defect is related to a substrate deficiency as described in hypercholesterinemic humans,8 a reduction in nitric oxide synthase activity, a diminished release, or an enhanced degradation of nitric oxide. However, a difference in vascular muscle sensitivity to nitric oxide appears to be an unlikely explanation as the response of smokers and nonsmokers to direct vascular muscle stimulation by sodium nitroprusside was similar.

Our data also provide indirect evidence for a disturbance of endothelial prostacyclin and/or endothelium-derived relaxing factor release on stimulation. Thus, the vasodilator response to low-dose endothelin-1 infusion, which has been related to endothelial release of both prostacyclin and endothelium-derived relaxing factor,33 was absent in smokers. Accordingly, the lack of vasodilator response to low-dose endothelin-1 infusion in smokers may represent a disturbed endothelial response of either or both endothelial vasodilating systems. The importance of prostacyclin in mediating this response in humans is stressed by the significant reduction, but not abolition, of endothelin-1–mediated forearm vasodilation after administration of acetylsalicylic acid.34

The present results are in line with recent studies investigating the vascular effects of stimulating nitric oxide release in smokers. Thus, coronary vasodilation in response to muscarinergic stimulation23 and brachial artery dilation in response to increased flow24 were markedly impaired in smokers. Taken together, these data suggest an impairment of endothelial control of vascular tone both under basal conditions and during stimulation.

The disturbance of nitric oxide–mediated control of basal vascular tone did not result in a long-term increase in vascular resistance in our subjects, suggesting that other mechanisms compensate for the lack of nitric oxide–mediated vasodilation. Indeed, vascular tone may even be reduced as suggested by epidemiological data showing reduced basal blood pressure in smokers,35,36 an observation consistent with the tendency of a lower systolic blood pressure in our smoking subjects. However, the mechanisms underlying this observation are not clear.

Smoking may also lead to a decrease in the atherogenic plasma high-density lipoprotein cholesterol fraction 2,37 and hypercholesterolemia is known to impair endothelial function.8,9 Our subjects had normal total and high-density plasma cholesterol concentrations. Therefore, this factor probably cannot account for
our findings, but we did not determine high-density lipoprotein subfractions.

**Short-Term Effects of Smoking**

Short-term smoking resulted in increases in heart rate and blood pressure, which have been attributed to both sympathoneuronal and sympathomediatory stimulation. The concomitant rise in plasma epinephrine and systolic blood pressure in our study is compatible with this contention, whereas the lack of an increase in plasma norepinephrine and in diastolic pressure, also observed by others, appears to disagree. This difference, however, may be related to the timing of blood sampling after smoking since plasma catecholamine concentrations were maximal at the end of a 10-minute smoking period and norepinephrine but not epinephrine concentrations declined within 10 minutes toward control values. Because we measured plasma catecholamines and blood pressure 5 minutes after smoking, this difference in timing may account for unchanged plasma norepinephrine concentrations and the small increase in diastolic compared with systolic blood pressure in our study. The dominance of sympathomediatory stimulation at the time of measurement through $\beta_2$-adrenoceptor–mediated muscle vasodilation may also explain the unchanged forearm vascular resistance in our subjects after smoking. Repetitive increases of sympathetic tone in smokers may also participate in atherogenesis since the uptake of atherogenic low-density lipoprotein is accelerated by epinephrine and norepinephrine at pathophysiological blood concentrations.

Short-term smoking did not affect the response to direct vascular muscle stimulation by sodium nitroprusside or blockade of nitric oxide synthesis by L-NMMA. The lack of an effect of short-term smoking on the vascular response to L-NMMA may appear surprising in view of the marked reduction of the basal L-NMMA effect in smokers. Possibly, the reduced L-NMMA response precludes further reductions after short-term smoking, or the cumulative impact of small and difficult-to-detect changes may be required for the development of the long-term disturbance of vascular response to L-NMMA. Letting nonsmokers smoke might answer this question, but the ethical limitations are obvious.

In contrast, the response pattern to endothelin-1 was changed: vasoconstriction was observed for the lowest dose, and the vasoconstrictor response to high doses was enhanced. We can only speculate about the underlying mechanisms, but a reduction of prostacyclin release after smoking might have unmasked the vasoconstrictor activity even of low-dose endothelin-1. A reduced endothelium–derived relaxing factor release may also be involved, but the unchanged response to L-NMMA after smoking argues somewhat against this possibility.

The enhanced vasoconstrictor response to endothelin-1 after smoking is not readily explained. In dogs, inhibition of endogenous nitric oxide synthesis augmented the vasoconstrictor property of endothelin-1, but short-term smoking did not change the effects of L-NMMA in our subjects. Moreover, the response to endothelin-1 was similar in smokers and nonsmokers, even though smokers had a reduced response to L-NMMA. Therefore, an altered balance between endothelium–derived vasodilating and vasoconstricting factors probably cannot explain our findings. Endothelin receptor stimulation is coupled to the phosphatidylinositol turnover pathway in an manner essentially similar to that of $\alpha_1$-adrenoceptors. Therefore, increased adrenergic vasoconstriction or an interaction of adrenergic and endothelin-1–induced stimulation of the phosphatidylinositol pathway may be important in mediating the enhanced vascular effect of endothelin-1 after smoking, but the exact mechanism(s) of this finding remain unclear.

A comparison of the vascular effects of norepinephrine and endothelin-1 before smoking confirms the greater vasoconstrictor potency of endothelin-1 in vivo. Although the difference in potency was not as great as previously suggested, regional differences exist, with mesenteric and renal vessels being most sensitive.

**Methodological Considerations**

We used L-NMMA as a tool to study endothelium–derived nitric oxide in humans. However, although L-NMMA inhibits nitric oxide synthesis in vitro, the acetylcholine-induced vascular relaxation was inhibited only by approximately 66%. Even though our highest dose of L-NMMA was twice that of the original study, we do not have data on the completeness of nitric oxide synthesis inhibition in our subjects. Recent data suggest that muscarinic receptor subtypes selectively mediate the release of nitric oxide and a putative, prostanoid endothelium–derived hyperpolarizing factor. Therefore, although an incomplete blockade of vascular acetylcholine effects by L-NMMA may be due to incomplete blockade of nitric oxide synthesis, it may also relate to the release of endothelium–derived hyperpolarizing factor. However, acetylsalicylic acid did not change the vasodilator response to acetylcholine in humans, suggesting that prostanoid release may not contribute significantly to the effects of acetylcholine in the human forearm. Regardless of the mechanism underlying the incomplete blockade of vascular acetylcholine effects, the reduced L-NMMA response in smokers probably cannot be explained by nonmaximal inhibition of nitric oxide synthesis only. Thus, the total L-NMMA effect in smokers was observed with the smallest dose (Fig 2). This contrasts with the finding in nonsmokers, in whom the L-NMMA response was dose dependent, and an additional effect of even higher doses of L-NMMA cannot be excluded. Such an effect, however, obviously would widen rather than narrow the gap between smokers and nonsmokers.

The decrease in forearm blood flow in response to L-NMMA infusion in our nonsmoking volunteers was somewhat greater than that in volunteers of previous studies (−54.2% versus −39.6% and −38%). This may be related to our use of a higher dose of L-NMMA and a different methodology for forearm blood flow measurement. Moreover, no information on the smoking status of the volunteers was provided in these studies, and the possible inclusion of smokers might also contribute to this difference.

We used norepinephrine infusions to control for an unspecified effect of smoking on vasoconstrictor stimuli. The reduced vasoconstrictor response to norepinephrine after smoking was unexpected but may be related to a reduced effect of exogenously administered norepinephrine in the presence of increased sympathetic.
stimulation and high α-adrenergic receptor occupancy by endogenous catecholamines.

The reproducible vasoconstrictor responses to nor-
epinephrine and endothelin-1 in smokers who waited for a similar time span before repeat drug infusions but did not smoke make an unspecific, time-related effect an unlikely explanation for the changes in vascular responses to norepinephrine and endothelin-1 observed after short-term smoking.

Potential Implications

The role of endothelin-1 in the regulation of vascular tone under normal and pathophysiological conditions is not defined. Therefore, the meaning of the enhanced vasoconstrictor response to exogenously administered endothelin-1 after smoking is not clear. Moreover, it is obviously unknown whether this finding would also apply to endogenously released endothelin-1. However, a potentially negative effect is conceivable in situations where endothelin-1 may be involved in the regulation of vascular tone, e.g., severe hypertension or congestive heart failure.49 Endothelin-1 may also play a role in the pathogenesis of coronary vasospasm.50 Because smoking has been identified as a strong risk factor for coronary vasospasm, it is tempting to speculate that smoking-induced enhancement of coronary vasoconstrictor effects of endothelin-1 may be causally involved. However, the introduction of endothelin-converting enzyme inhibitors or specific receptor antagonists has to be awaited to clarify such issues.

Endothelin-1 also has mitogenic activity and may be involved in the development of atherosclerotic vascular changes.16 Whether smoking interferes with this effect has not been investigated.

Prostacyclin and endothelin-derived nitric oxide have synergistic effects, resulting in relaxation of vascular smooth muscle and inhibition of adhesion and aggregation of blood platelets.4,55 These systems thereby represent an important endothelial defense mechanism against bloodborne cells and chemicals. Consequently, a disturbance of this defense mechanism may be an important factor leading to the chain of events that ultimately result in the development of atherosclerotic vascular changes.3 The reduced nitric oxide–dependent component of basal vascular tone and the impaired endothelin-dependent vasodilator response found in this and other studies5,58 in smokers may therefore not only reflect a functional defect of the endothelium related to the regulation of vascular tone but, more important, also indicate a loss of its normal antiatherosclerotic functions. Several lines of evidence suggest also that smoking interferes with prostacyclin metabolism. A reduction in prostacyclin production has been described in cultured endothelial cells and in the thoracic aorta of rats exposed to tobacco smoke,56 in umbilical arteries of babies born to smoking mothers, and in long-term smokers after smoking.58 An increased urinary excretion of the prostacyclin metabolite 2,3-
dinor-6-keto-prostaglandin-F1α, together with an increase in thromboxane A2 metabolites in healthy, long-term smokers has been taken as an index for an enhanced platelet–vessel wall interaction.57 Although the increase in prostacyclin metabolites is not undisput-
ed,58 the data are consistent with respect to an increase in platelet-derived thromboxane A2 metabolites indicat-
ing platelet activation in smokers. Thus, a weakening of the normal endothelial antiatherosclerotic defense line, as possibly reflected by the functional changes of the endothelium, together with smoking-induced platelet activation, enhanced endothelin-1–mediated vasoconstriction, and catecholamine-induced stimulation of atherogenic low-density lipoprotein uptake may ultimately contribute to the development of atherosclerotic vascular changes.

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Diminished vascular response to inhibition of endothelium-derived nitric oxide and enhanced vasoconstriction to exogenously administered endothelin-1 in clinically healthy smokers.

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