Mechanisms Responsible for Sympathetic Activation by Cigarette Smoking in Humans

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Background The pressor and tachycardic effects of cigarette smoking are associated with an increase in plasma catecholamines, suggesting the dependence of these effects on adrenergic stimulation. Whether the stimulation occurs at a central or a peripheral level and whether reflex mechanisms are involved is unknown.

Methods and Results In nine normotensive healthy subjects (age, 33.0±3.5 years, mean±SEM), we measured blood pressure (Finapres device), heart rate (ECG), calf blood flow and vascular resistance (venous occlusion plethysmography), plasma norepinephrine and epinephrine (high-performance liquid chromatography assay), and postganglionic muscle sympathetic nerve activity (microneurography from the peroneal nerve) while subjects were smoking a filter cigarette (nicotine content, 1.1 mg) or were in control condition. Cigarette smoking (which raised plasma nicotine measured by high-performance liquid chromatography from 1.0±0.9 to 44.2±7.1 ng/mL) markedly and significantly increased mean arterial pressure (+13.2±2.3%), heart rate (+30.3±4.7%), cef blood flow and vascular resistance (+12.1±4.9%), plasma norepinephrine (+34.8±7.0%), and plasma epinephrine (+90.5±39.0%). In contrast, muscle sympathetic nerve activity showed a marked reduction (integrated activity −31.8±5.1%, P<.01). The reduction was inversely related to the increase in mean arterial pressure (r=−.67, P<.05), but the slope of the relation was markedly less (−54.1±7.5%, P<.05) than that obtained by intravenous infusion of phenylephrine in absence of smoking. The hemodynamic and neurohumoral changes were still visible 30 minutes after smoking and occurred again on smoking a second cigarette. Sham smoking was devoid of any hemodynamic and neurohumoral effect.

Conclusions These data support the hypothesis that in humans the sympathetic activation induced by smoking depends on an increased release and/or a reduced clearance of catecholamines at the neuroeffector junctions. Central sympathetic activity is inhibited by smoking, presumably via a baroreceptor stimulation triggered by the smoking-related pressor response. The baroreflex is impaired by smoking, however, indicating that partial inability to reflexly counteract the effect of sympathetic activation is also responsible for the pressor response. (Circulation. 1994;90:248-253.)

Key Words • smoking • blood pressure • nervous system • baroreflex

Cigarette smoking is accompanied by a marked and prolonged increase in heart rate and blood pressure.1-5 These hemodynamic changes are markedly attenuated by α- and β-adrenergic blockade.3,4,6,7 They are also associated with a marked and prolonged increase in plasma norepinephrine and epinephrine,6,8,9 which has prompted the hypothesis that the mechanisms responsible for the pressor and tachycardic responses have an adrenergic nature.

After the report of preliminary data from our group,10 Niedermayer et al11 have recently reported that in eight habitual smokers smoking caused a reduction rather than an increase in postganglionic muscle sympathetic nerve traffic as assessed by microneurography. However, these findings are difficult to interpret because these observations are in conflict with those reported by the same authors in a series of 10 habitual smokers in whom no smoking-dependent alterations in sympathetic traffic were observed.12 Furthermore, at variance from a large number of studies,6,8,9 smoking in these subjects did not cause an increase in plasma norepinephrine,11 thus failing to reproduce the smoking-dependent adrenergic activation.

The present study was planned to provide information on this issue. To this aim, we studied habitual smokers in whom smoking was accompanied by a marked increase in blood pressure, heart rate, and plasma norepinephrine. We examined the effect of smoking on both sympathetic traffic and on baroreceptor-sympathetic reflex to determine whether, in the presence of a smoking-induced adrenergic activation, central and reflex sympathetic mechanisms are involved.

Methods

We studied nine normotensive healthy subjects (eight men, one woman) whose mean age was 33.0±3.5 years (mean±SEM; range, 21 to 48 years). The subjects were all habitual cigarette smokers (>10 cigarettes daily). They agreed to participate in the study after being informed of its nature and purpose. The protocol of the study (see below) was approved by the ethical committee of our institution.

Hemodynamic, Microneurographic, and Humoral Measurements

Arterial blood pressure was measured by a digital photoplethysmographic device (Finapres, Ohmeda 2300) capable of providing beat-to-beat systolic and diastolic values similar to those obtained intra-arterially under conditions ranging from generalized vasodilatation to generalized vasoconstriction.13,14 Heart rate was monitored by a cardiotachometer triggered by
the R wave of an ECG lead. Calf blood flow (mL/min per 100 g) was measured four times per minute by venous occlusion plethysmography (Hokanson, EC4), using a mercury-in-Silastic strain gauge applied around the calf of the leg contralateral to the leg used for microneurography (see below). The strain gauge was placed 5 to 6 cm below the fibular head, and the measurements were made at constant room temperature (23°C to 24°C), with the foot circulation excluded by a cuff positioned at the ankle and inflated at a suprasystolic pressure. Calf vascular resistance was calculated as the ratio of mean arterial pressure (diastolic blood pressure plus one third of pulse pressure) to blood flow and expressed in arbitrary units.

Multunit recording of postganglionic sympathetic nerve traffic was obtained from the peroneal nerve of the leg contralateral to the one used for calf blood flow measurements. The nerve was impaled posteriorly to the fibular head with a tungsten microelectrode of a diameter of 200 µm in the shaft, tapering to a 1- to 5-µm uninsulated tip. A reference electrode positioned subcutaneously 1 to 3 cm from the recording electrode served as ground. The nerve signal was amplified ×70 000, fed through a band-pass filter (700 to 2000 Hz), and integrated with a custom nerve-traffic analysis system (Bioengineering Department, University of Iowa). Integrated nerve activity was monitored by a loudspeaker, displayed on a storage oscilloscope (model 511 A, Tektronix), and recorded, together with blood pressure, heart rate, arterial blood flow and respiratory movements (strain-gauge pneumograph), on a Gould 3800 RS paper recorder. Sympathetic bursts were identified by inspection of the mean voltage neurogram and proved to be directed to muscle districts by currently accepted criteria (increase in traffic with Valsalva maneuver, no change in traffic with gentle stroking of the skin distal to the recording site, no response to arousal, etc.16-18 Muscle sympathetic nerve activity (MSNA) was calculated as burst frequency over time (bursts per minute) and/or integrated activity (bursts per minute multiplied by mean burst amplitude, expressed in arbitrary units). Quantification of MSNA by these methods has been shown to have a 5% intraobserver variability.17

Plasma norepinephrine and epinephrine were measured by high-performance liquid chromatography19 from blood samples taken from an antecubital vein. The samples were drawn into a syringe and collected into ice-chilled tubes containing EGTA glutathione. The tubes were promptly centrifuged and stored at −70°C until assayed. The blood samples were used also to measure plasma renin activity and plasma vasopressin by radioimmunoassay. The methods used for these measurements have been described in detail in previous papers.20 In eight subjects, measurements included plasma nicotine concentrations. This was done by high-performance liquid chromatography, with an instrument (Aerograph 1400) equipped with a nitrogen phosphorus detector.21

Protocol

All subjects were asked to abstain from smoking and coffee and alcohol consumption during the 48 hours preceding the study. The study proper was conducted in the morning, 1 to 2 hours after a light breakfast, according to the following protocol: (1) The subject was put in the supine position, and the various measuring devices plus the venous cannula were set in position. (2) After 40 minutes of rest, continuous blood pressure, heart rate, and MSNA were obtained over a 15-minute period. Calf blood flow was measured during the last 2 minutes, and venous blood was withdrawn during the last 30 seconds. (3) The subject was asked to smoke a cigarette of a filtered type containing 1.1 mg nicotine. Puffing habits were left uncontrolled, but the subject was required to finish the cigarette within 5 minutes. During this period, blood pressure, heart rate, and MSNA were continuously recorded; calf blood flow was measured during the last 2 minutes, and venous blood was withdrawn during the last 30 seconds. (4) Blood pressure, heart rate, and MSNA measurements were continued for 15 minutes (recovery), after which the sequence of a control, a smoking, and a recovery period was repeated according to a procedure identical to the previous one.

In five subjects, the protocol described above was performed a second time except that the cigarette was replaced with a drinking straw provided with a filter to simulate the respiratory and behavioral activity of smoking (sham smoking).

In a further experimental session on seven subjects, the effects of smoking on blood pressure, heart rate, and MSNA were compared with those induced by a 5-minute intravenous infusion of phenylephrine (1.2 µg/min per kilogram body weight) capable of producing a pressor response similar to that induced by cigarette smoking.24 Phenylephrine was infused after a 30-minute recovery from smoking and a subsequent 15-minute control period.

Data Analysis

In each subject, systolic blood pressure, diastolic blood pressure, mean blood pressure, heart rate, and microneurographic data were averaged for each minute of the recording period. These data were further averaged for the 15 minutes preceding smoking (control) and the 5 minutes of smoking. Averages were also obtained of the calf blood flows and vascular resistances before and during smoking. Data from individual subjects were averaged for the group as a whole and expressed as means±SEM. The statistical significance of the changes induced by smoking was assessed by two-way ANOVA. The paired t test with Bonferroni’s correction was used to locate the difference between the smoking and presmoking values, as well as between changes induced by smoking the first and the second cigarette. The same statistical analysis was used to locate the difference between the smoking and phenylephrine data. The Spearman analysis was used to assess the correlation between the various changes induced by smoking. A value of P<.05 was taken as the level of statistical significance.25

Results

As shown in Fig 1, smoking the first cigarette caused an increase in systolic blood pressure, diastolic blood pressure, and heart rate. Calf blood flow was unchanged by smoking, whereas calf vascular resistance was markedly increased. These hemodynamic changes were accompanied by a marked elevation in plasma norepinephrine and epinephrine. This was paralleled by a marked MSNA reduction, which was equally apparent when calculated as units and as bursts per minute (see “Methods”). The reduction in MSNA was inversely correlated with the increase in mean arterial pressure and calf vascular resistance (r = −.67, P<.05; and r = −.83, P<.01; respectively). On the other hand, no significant correlation was found between changes in MSNA and in plasma norepinephrine (r = .26) or epinephrine (r = .31). An example of the MSNA reduction in one subject is illustrated in Fig 2.

Fig 3 shows the blood pressure, heart rate, and MSNA values throughout the whole study. The blood pressure and heart rate increase and the MSNA reduction induced by the first cigarette were immediate and sustained. The changes were only partially reversed after completion of smoking and still visible at the end of the recovery and the second control period. Smoking the second cigarette induced blood pressure, heart rate, and MSNA effects qualitatively similar to those of the first one. This was the case also for calf hemodynamics and plasma catecholamines (Fig 4). Smoking the first cigarette did not affect ventilation rate and plasma renin activity, but it caused a consistent increase in plasma
vasopressin. This was the case also for smoking a second cigarette (Table 1).

As shown in Table 2, sham smoking was devoid of any significant effect on ventilation rate, hemodynamic variables, MSNA, and humoral variables. Smoking the first or the second cigarette strikingly increased the plasma nicotine levels in contrast with the absence of any increase during sham smoking (Tables 1 and 2).

Fig 5 compares the effects on blood pressure, heart rate, and MSNA of cigarette smoking and phenylephrine infusion. As expected, phenylephrine infusion increased blood pressure and decreased heart rate and MSNA, both when expressed in units and in number of bursts per minute. Although they caused similar blood pressure increases, phenylephrine caused a reduction in heart rate while smoking caused an increase. Furthermore, the reduction in MSNA associated with phenylephrine was significantly greater ($P<.05$) than that associated with smoking.

**Discussion**

In our subjects, smoking was accompanied by an immediate, marked, and sustained increase in blood
pressure, heart rate, and calf vascular resistance. These responses were associated with an increase in plasma norepinephrine and epinephrine, confirming previous observations.6,8,9 There was, however, no increase in postganglionic muscle sympathetic nerve traffic, which, on the contrary, showed an immediate, marked, and sustained reduction specular to the blood pressure change. This suggests that the adrenergic activation associated with smoking does not have a central origin, nor does it depend on stimulation of ganglionic sympathetic transmission. It suggests rather that smoking acts at peripheral sympathetic sites to (1) enhance catecholamine release from chromaffin tissues and/or (2) reduce the reuptake and the overall clearance of these substances.

The mechanisms involved in the central sympathoinhibition of smoking are not explained by our data. Studies in animals have reported that local injection of nicotine in several brain-stem sites is accompanied by hypotension and bradycardia,26,27 suggesting that the main product of smoking may inhibit centrally sympathetic activity. In other studies, however, microinjection of nicotine in a number of other brain-stem areas has produced pressor rather than depressor effects.28,29 Furthermore, in our patients the degree of sympathetic inhibition induced by smoking showed an inverse relation with the magnitude of the pressor and vasoconstrictor responses. This suggests that, rather than being directly due to smoking (either via nicotine or via other smoking constituents), this inhibition is mediated by a baroreflex mechanism; ie, it is due to the baroreceptor stimulation brought about by the smoking-dependent peripheral adrenergic stimulation and blood pressure rise.

In the above context, however, our study provides additional new information. The slope of the sympathetic inhibition was markedly less for the pressor effect of smoking than for the pressor effect of phenylephrine, indicating a smoking-associated impairment of the baroreflex. Because smoking is accompanied by a reduction in arterial compliance,30 we can speculate that this impairment is due to a reduction in the sensitivity of stretch "sensors" such as the baroreceptors. We can speculate also that a nicotine-dependent stimulation of arterial chemoreceptors31 impairs the baroreflex.32 Finally, a third possibility is an influence of nicotine (or other component of smoking) on the integrating baroreflex centers, although this would imply a central as well as a peripheral action of smoking. Regardless of the mechanisms, however, it is clear from our data that there are probably two reasons for the sympathoexcitatory effects of smoking in humans, ie, (1) a peripheral adrenergic stimulation and (2) a partial loss of the baroreflex ability to counteract it.

Other aspects of our study deserve to be mentioned. First, in our subjects the hemodynamic, humoral, and nerve activity changes associated with smoking were still partly evident 30 minutes after the first smoking episode was completed. This provides further evidence

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**Table 1. Effects of Cigarette Smoking on Ventilatory Rate, Humoral Variables, and Plasma Nicotine Levels**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>1st Cigarette Smoking</th>
<th>Control</th>
<th>2nd Cigarette Smoking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventilatory rate, cycles/min</td>
<td>15.4±1.1</td>
<td>15.1±1.2</td>
<td>14.7±0.9</td>
<td>15.2±1.1</td>
</tr>
<tr>
<td>Plasma renin activity, ng/mL per hour</td>
<td>0.7±0.1</td>
<td>0.6±0.1</td>
<td>0.6±0.1</td>
<td>0.5±0.1</td>
</tr>
<tr>
<td>Plasma vasopressin, pg/mL</td>
<td>0.9±0.2</td>
<td>2.1±0.4*</td>
<td>1.2±0.2</td>
<td>2.5±0.3*</td>
</tr>
<tr>
<td>Plasma nicotine, ng/mL</td>
<td>1.0±0.9</td>
<td>44.2±7.1†</td>
<td>8.1±3.8</td>
<td>48.9±6.2†</td>
</tr>
</tbody>
</table>

Values are means±SEM. n=9 (except for plasma nicotine, n=5).

*P<.05; †P<.01 versus control.

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**Fig 4.** Bar graphs showing mean arterial pressure (MAP), heart rate (HR), calf blood flow (CBF), calf vascular resistance (CVR), plasma norepinephrine (NE), plasma epinephrine (E), and muscle sympathetic nerve activity (MSNA), expressed as integrated activity (left, units) and as burst frequency (right, bursts per minute [B/min]) before (open histograms) and during (dashed histograms) smoking the first cigarette and before (open histograms) and during (dashed histograms) smoking the second cigarette. Values are shown as means±SEM. *P<.05; **P<.01.
that the cardiovascular effects of smoking are long-lasting and that in a heavy smoker (i.e., a subject smoking 2 to 3 cigarettes per hour or 20 to 40 cigarettes per day) the cardiovascular system is likely to be under the permanent hemodynamic, humoral, and neural effects of smoking. Second, smoking was associated with no change in plasma renin activity but with a marked increase in plasma vasopressin levels. This may suggest that the hemodynamic responses to smoking may depend also on a nonadrenergic mechanism, such as increased circulating vasopressin. Because vasopressin infusion inhibits MSNA, an increase in vasopressin might partly account for the smoking-dependent reduction in sympathetic firing, although persistence of this reduction 30 minutes after smoking the first cigarette when baseline plasma vasopressin level was restored excludes this as a major factor (see Table 1).

Finally, microneurography is the only technique that allows the effect of smoking on sympathetic nerve activity to be monitored directly and dynamically. However, this technique does not allow the examination of sympathetic neural activity to extend to splanchnic, renal, and other important vascular districts. Thus, whether smoking affects sympathetic activity in these districts as it does in skeletal muscle (i.e., whether its sympathetic modulation is homogeneous or heterogeneous) remains to be clarified.

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**References**


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