Mechanisms Underlying Cerebrovascular Effects of Cigarette Smoking in Rats In Vivo

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Background and Purpose—The effects of acute smoking on cerebral circulation are controversial. This study was designed (1) to clarify any differences between the effects of cigarette smoking and nicotine infusion and between the effects of single- and multiple-cigarette smoking on cerebral vessels and (2) to probe the mechanism(s) underlying the vascular responses.

Methods—In pentobarbital-anesthetized, mechanically ventilated Sprague-Dawley rats, pial vessel diameters were measured with the use of a cranial window preparation. We studied the effects of (1) 60 puffs per minute of mainstream cigarette smoke from cigarettes having 2 nicotine levels (0.1 and 1 mg per cigarette), (2) administration of nicotine (0.05 mg per body IV), and (3) repeated smoking (four 1 mg nicotine–containing cigarettes at 30-minute intervals) (n=6 each).

Results—Inhalation of smoke from a 0.1 or 1 mg nicotine–containing cigarette for 1 minute caused pial arterioles to constrict at 30 seconds (7.2% and 7.3%, respectively) and then to dilate (peak at 5 to 10 minutes; 4.6% and 17.9%, respectively). Nicotine infusion caused pial vasodilation (35.7%) without an initial vasoconstriction. Repeated smoking suppressed the pial vasodilation but not the initial vasoconstriction. The vasodilation induced by a single cigarette was greatly inhibited by pretreatment with mecamylamine or glibenclamide and attenuated by propranolol or \( N^\text{v} \)-nitro-L-arginine methyl ester; the initial vasoconstriction was inhibited by seratrodast, a thromboxane A\(_2\) receptor antagonist (n=6 in each case).

Conclusions—Single-cigarette smoking had a significant biphasic effect on cerebral arteriolar tone. The vasodilation was attenuated by repeated smoking. The vasodilation is most likely an effect of nicotine, at least in part mediated via sympathetic activation, NO production, and K\(_+\) channel activation. The vasoconstriction is partially due to thromboxane A\(_2\) induced by cigarette smoke. (Stroke. 1998;29:1656-1665.)

Key Words: cerebral vessels • cigarette smoking • microcirculation • nicotine • rats

Cigarette smoking is known to be associated with atherosclerosis and to be an important risk factor for stroke.\(^1\)-\(^3\) Although chronic cigarette smoking has been reported to reduce cerebral blood flow (CBF),\(^4\)-\(^5\) acute inhalation of cigarette smoke or administration of nicotine has been reported to increase,\(^6\) to maintain,\(^7\) or to decrease\(^8\) CBF levels in smokers. These apparent discrepancies between the effects of chronic\(^4\)-\(^5\) and acute smoking\(^6\) and among acute studies\(^6\)-\(^8\) could arise from many factors, including the dose of cigarette smoke or nicotine, the individual’s smoking history, and different timing of the measurements. Since chronic smokers in effect undergo repeated acute inhalation of mainstream smoke, it is crucial to clarify the acute influence of cigarette smoking on cerebral vasculature.

Mainstream cigarette smoke has been said to contain 4000 or more constituents (eg, nicotine, tar, phenol, acetic acid, CO, CO\(_2\), NO, and NO\(_2\)).\(^9\) The influence of cigarette smoking on cerebral vasculature seems likely to be a net effect of not only nicotine but also the other constituents of mainstream smoke. In isolated coronary arteries, cigarette smoke extract produces a biphasic action on vascular tone (contraction followed by relaxation).\(^10\) In addition to the potential biophysical differences between coronary and cerebral arteries, in vivo cigarette smoking is quite different from the in vitro situation. In vitro studies bypass the airways and eliminate the filtering action of the lungs. The aims of the present study were to investigate the acute effects of cigarette smoking on cerebral vessels in rats in vivo with the use of the cranial window technique and to elucidate the mechanisms involved. We tested the hypothesis that the effects of cigarette smoking on tone in the cerebral vasculature are not same as the effects of nicotine and that these effects are not only by means of nicotinic receptors. In addition, we investigated the effects of multiple-cigarette smoking on the pial microcirculation by studying repeated inhalation. We also tested the hypothesis that multiple-cigarette inhalation attenuates the effects of single inhalation on cerebral vasculature.
Materials and Methods

Experimental Animals

We studied 86 male Sprague-Dawley rats weighing 350 to 400 g. Experimental protocols were approved by our institution’s animal care committee.

Each rat was anesthetized (pentobarbital sodium, 50 mg/kg body weight IP) and mechanically ventilated through a tracheostomy tube by a ventilator (600; No. KN-56, Natsume Seisakusho Co Ltd) using room air supplemented with oxygen. Tidal volume was adjusted to maintain PaCO_2 between 35 and 40 mm Hg at the beginning of each experiment by a pressure-limited system. Supplemental pentobarbital was administered intravenously by continuous infusion at 4 mg·kg⁻¹·h⁻¹.

The femoral artery was cannulated for the continuous measurement of arterial blood pressure and to provide blood samples for the determination of arterial blood gas tensions, pH, glucose, and serum electrolytes. The femoral vein was cannulated for administration of fluid and drugs. Body temperature was maintained between 37°C and 38°C by means of a heating blanket.

A closed cranial window was used for observation of the pial microcirculation; the head was fixed in the splinh position. The scalp was retracted, a 3×2-mm-diameter hole was made in the bone over the right parietal cortex, and the dura was opened carefully. A polypropylene ring with a fitted glass coverslip was placed over the space under the window containing artificial cerebrospinal fluid to maintain a constant hole and secured with dental acrylic.

The pial views obtained in these experiments were stored on videotape (with a time record) for later playback and analysis. The diameters of 3 pial arterioles and 3 pial venules (base diameter, 21 to 75 μm and 23 to 51 μm, respectively) were measured using a videomicroscope (model VM-20, Olympus) on a television monitor attached to a microscope (model SZH-10, Olympus). The value of the percent changes in pial vessel diameter was used in the statistical analysis. Mean arterial blood pressure (MAP) and heart rate (HR) were continuously monitored.

Experimental Protocols

In protocol 1, we tested whether the main effect of cigarette smoking on pial vessels was due to nicotine. We evaluated the effects of mainstream smoke on pial vessels and compared them with the effects of intravenous nicotine. Two different commercial cigarettes, a high nicotine–containing cigarette (nicotine, 1 mg; tar, 13 mg; Marlboro, Philip Morris) and a low nicotine–containing cigarette (nicotine, 0.1 mg; tar, 1 mg; Next, Philip Morris) were used to provide mainstream smoke in 6 rats each. Each animal inspired mainstream smoke for 1 minute through its tracheal cannula (by way of the ventilator). The gas inlet of the ventilator was divided into 2 tubes. A lighted cigarette was fitted into one of them, and oxygen supply tube was connected to the other one. Therefore, a mixture of mainstream smoke for 1 minute through its tracheal cannula (by way of the ventilator). The gas inlet of the ventilator was divided into 2 tubes. A lighted cigarette was fitted into one of them, and oxygen supply tube was connected to the other one. Therefore, a mixture of mainstream smoke, air, and oxygen was inspired through the ventilator. Nicotine (0.05 mg per rat in 0.5 mL saline) (Sigma Chemical Co) was infused intravenously over 1 minute in 6 rats. We chose 0.05 mg nicotine for infusion because in our pilot study this dose induced an increase in MAP that was similar to that caused by smoking a 1 mg nicotine–containing cigarette for 1 minute.

In protocol 2, we investigated the vasodilator mechanisms involved in the effects of mainstream smoking on pial vessels. Four kinds of drugs were used for pretreatment in 6 rats each. Nicotine receptors were blocked with mecamylamine (0.7 mg/kg IV) (Sigma); sympathetic β-adrenoceptors, with propranolol (1 mg/kg IV) (Wako Pure Chemical); ATP-sensitive K⁺ channels, with glibenclamide (20 mg/kg IV) (Sigma); and NO synthase, with N‘-nitro-L-arginine methyl ester (L-NAME, 10 mg · kg⁻¹ · h⁻¹, intravenous infusion) (Sigma). The effect of smoking a 1 mg nicotine–containing cigarette on pial vessels was investigated as in protocol 1. Mecamylamine, propranolol, and glibenclamide were given 5 minutes before smoking. L-NAME was started 30 minutes before smoking.

In protocol 3, we investigated the vasoconstrictor mechanisms involved in the effects of mainstream smoking on pial vessels. During the blockade of thromboxane (Tx) A₂ receptors with serotonin (5 mg/kg IV) (Takeda Chemical), the effect of smoking a 1 mg nicotine–containing cigarette on pial vessels was investigated in 6 rats as in protocol 1. Seratrodast was administered 5 minutes before smoking. The arterial concentration of TxB₂, (a stable metabolite of TxA₂) was measured at baseline and just after the smoking of a 1 mg nicotine–containing cigarette (n=6) or intravenous nicotine administration (0.05 mg per rat, n=6).

In protocol 4, the same preparation was used in 6 rats, and mainstream smoke from four 1 mg nicotine–containing cigarettes was inhaled for 1 minute each time at 30-minute intervals (repeated smoking). Measurements were made as in protocol 1 when the fourth dose was inhaled.

In protocol 5, venous nicotine concentrations were measured at baseline, immediately after, and 30 minutes after 1-minute inhalation of smoke from a 1 mg nicotine–containing cigarette or intravenous nicotine administration (0.05 mg per rat), as in protocol 1 (n=4 each).

All drug solutions were freshly prepared on the day of the experiment.

Data Analysis

All variables (time-dependent effects of smoking, of nicotine injection, of repeated smoking, and of smoking after pretreatment with mecamylamine, propranolol, L-NAME, glibenclamide, or seratrodast) were examined by a 1-way ANOVA for repeated measurements followed by the Scheffé F test for post hoc comparison. The effects of mecamylamine, propranolol, L-NAME, glibenclamide, seratrodast, or repeated smoking on the smoking-induced pial vessel changes were compared with control values (no pretreatment) by a 2-way ANOVA. An unpaired t test was used to examine the differences between 2 groups. A paired t test was used to determine the significance of changes in the plasma TxB₂ level induced by cigarette smoking or intravenous nicotine administration. Significance was set at P<0.05. All values are presented as mean±SEM.

Results

Effects of Smoking or Nicotine Infusion on Pial Vessels and Physiological Values (Protocol 1)

Acute single-cigarette smoking and nicotine infusion both caused significant changes in pial arteriolar and venular diameter and MAP (Figure 1). Smoking (both 0.1 mg and 1 mg nicotine–containing cigarettes) caused pial arterioles to constrict at 30 seconds; they then dilated, with the peak occurring at 5 to 10 minutes and being dose dependent (in terms of nicotine and tar). Nicotine infusion caused a vasodilation of pial arterioles without an initial vasoconstriction. Venular vasodilation was induced at 2 to 10 minutes after the smoking of a 1 mg nicotine–containing cigarette or nicotine infusion. MAP increased significantly during smoking or nicotine infusion but decreased to baseline immediately after the inhalation or infusion was stopped. Thus, the peak vasodilation associated with cigarette smoking occurred some 4 minutes or more after the peak increase in MAP, at a time when MAP was at or below baseline values. In terms of MAP, there was no significant difference between these 2 groups throughout the experiment. There was no marked...
change in HR after smoking or nicotine infusion, except for a decrease at 2 minutes after nicotine infusion (Figure 1).

Arterial blood gas tensions, pH, serum electrolytes, and body temperature did not change significantly throughout the experiment, although there were significant increases in blood glucose at 15 minutes after smokinga1m gnicotine–containing cigarette and nicotine infusion (Table 1).

### Effects of Antagonistic Drugs on the Vasodilator Response to Smoking a 1 mg Nicotine–Containing Cigarette (Protocol 2)

Blockade of autonomic ganglia (nicotine receptor blocking) with mecamylamine completely inhibited the smoking-induced pial arteriolar vasodilation (Figure 2A). The small, but significant, initial vasoconstriction was not reduced by mecamylamine; in fact, it was prolonged (it persisted for 30 minutes).

Propranolol attenuated the smoking-induced arteriolar dilation but not the initial vasoconstriction observed during cigarette smoking (Figure 2B).

L-NAME partially attenuated the smoking-induced arteriolar dilation at 5 minutes but not the initial vasoconstriction observed during cigarette smoking (Figure 2C). Pial arterioles showed a continuous smoking-induced dilation from 2 to at least 60 minutes as a result of L-NAME pretreatment.

Glibenclamide completely inhibited the smoking-induced arteriolar dilation but not the initial vasoconstriction (Figure 2D). Baseline pial vessel diameters did not change as a result of propranolol, L-NAME, and glibenclamide administration, although they were dilated 8.2% by mecamylamine pretreatment.

Physiological variables, including arterial pH, PacO2, PaO2, Na+, K+, and body temperature, all remained stable in the presence of the above antagonists, but there was a decrease in pH (from 7.37 to 7.33) at 60 minutes in the presence of L-NAME. However, blood glucose was increased by propranolol and decreased by glibenclamide (Table 2). The changes in MAP and HR during protocol 2 are summarized in Table 3. MAP decreased with mecamylamine and increased with L-NAME.

### Effects of an Antagonistic Drug on the Pial Vasconstrictor Response to Smoking a 1 mg Nicotine–Containing Cigarette and on Plasma TxB2 Concentration (Protocol 3)

The TxA2 receptor blocker, seratrodast, completely inhibited the smoking-induced initial pial arteriolar vasoconstriction. The subsequent vasodilation was not increased in amplitude by seratrodast, but it was prolonged (it persisted for 60 minutes) (Figure 3). Baseline pial vessel diameters did not change as a result of seratrodast administration. Physiological parameters did not change throughout the experiment, and the changes in MAP and HR are shown in Table 3.

Smoking a 1 mg nicotine–containing cigarette caused the arterial TxB2 concentration to increase significantly from 48.7±6.0 to 110.3±18.4 pg/mL (P<0.01), but intravenous nicotine administration did not affect the concentration (from 37.8±6.5 to 34.9±2.9 pg/mL) (Figure 4). The second mea-
TABLE 1. Changes in Physiological Parameters During and After Smoking or Nicotine Infusion

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>1 min</th>
<th>5 min</th>
<th>15 min</th>
<th>60 min</th>
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<tr>
<td>pH</td>
<td></td>
<td></td>
<td></td>
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<td>1 mg nicotine cigarette</td>
<td>7.42±0.01</td>
<td>7.41±0.01</td>
<td>7.42±0.01</td>
<td>7.42±0.02</td>
<td>7.39±0.04</td>
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<td>7.40±0.02</td>
<td>7.39±0.02</td>
<td>7.38±0.02</td>
<td>7.37±0.02</td>
<td>7.37±0.01</td>
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<td>Nicotine infusion</td>
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<td>7.38±0.02</td>
<td>7.38±0.02</td>
<td>7.39±0.01</td>
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<td>PaCO2, mm Hg</td>
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<tr>
<td>1 mg nicotine cigarette</td>
<td>35.4±0.7</td>
<td>36.6±1.0</td>
<td>36.5±1.7</td>
<td>36.0±2.3</td>
<td>38.9±1.1</td>
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<td>0.1 mg nicotine cigarette</td>
<td>37.2±1.1</td>
<td>38.9±1.0</td>
<td>40.3±1.6</td>
<td>40.2±1.5</td>
<td>40.2±1.0</td>
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<td>Nicotine infusion</td>
<td>35.9±1.2</td>
<td>37.8±2.3</td>
<td>38.3±1.8</td>
<td>38.0±2.0</td>
<td>35.4±1.2</td>
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<td>PaO2, mm Hg</td>
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<td>1 mg nicotine cigarette</td>
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<td>261±9.0</td>
<td>246±19</td>
<td>238±20</td>
<td>235±15.0</td>
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<td>0.1 mg nicotine cigarette</td>
<td>294±15</td>
<td>288±15</td>
<td>297±20</td>
<td>293±17</td>
<td>279±10</td>
</tr>
<tr>
<td>Nicotine infusion</td>
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<td>267±21</td>
<td>246±26</td>
<td>247±28</td>
<td>263±12</td>
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<td>Na⁺, mEq/L</td>
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<td>1 mg nicotine cigarette</td>
<td>144±1</td>
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<td>144±1</td>
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<tr>
<td>0.1 mg nicotine cigarette</td>
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<tr>
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<td>K⁺, mEq/L</td>
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<tr>
<td>1 mg nicotine cigarette</td>
<td>4.2±0.2</td>
<td>4.0±0.1</td>
<td>4.1±0.1</td>
<td>3.9±0.1</td>
<td>3.8±0.2</td>
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<td>0.1 mg nicotine cigarette</td>
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<td>4.2±0.1</td>
<td>4.3±0.1</td>
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<tr>
<td>Nicotine infusion</td>
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<td>3.8±0.1</td>
<td>4.2±0.1</td>
<td>3.7±0.1</td>
<td>3.7±0.2</td>
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<td>Glucose, mg/dL</td>
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<td></td>
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<td>1 mg nicotine cigarette</td>
<td>145±6</td>
<td>152±9</td>
<td>161±5</td>
<td>171±14*</td>
<td>155±5</td>
</tr>
<tr>
<td>0.1 mg nicotine cigarette</td>
<td>146±17</td>
<td>151±15</td>
<td>161±16</td>
<td>167±19</td>
<td>167±12</td>
</tr>
<tr>
<td>Nicotine infusion</td>
<td>139±10</td>
<td>142±11</td>
<td>182±12*</td>
<td>178±12*</td>
<td>154±9</td>
</tr>
<tr>
<td>BT, °C</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1 mg nicotine cigarette</td>
<td>37.2±0.2</td>
<td>37.2±0.2</td>
<td>37.2±0.2</td>
<td>37.1±0.1</td>
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<tr>
<td>0.1 mg nicotine cigarette</td>
<td>37.2±0.2</td>
<td>37.2±0.2</td>
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<td>Nicotine infusion</td>
<td>37.1±0.1</td>
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<td>37.2±0.1</td>
<td>37.2±0.2</td>
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</tbody>
</table>

BT indicates body temperature. Values are mean±SEM.

*P<0.01 compared with baseline.

Discussion

The major finding of the present study was that acute inhalation of mainstream cigarette smoke produced a significant biphasic change in the diameter of cerebral arterioles. Smoke inhalation caused pial arterioles to constrict at 30 seconds, followed by a dose-related vasodilation that peaked at 5 to 10 minutes; MAP increased only during the inhalation and returned to baseline 4 minutes or more before arteriolar diameter reached its peak. Since the increase in arteriolar diameter was not concomitant with the increase in MAP, the changes in arteriolar diameter seem likely to be due to a direct effect of smoking on vascular tissue. Nicotine infusion caused a vasodilation of pial vessels without an initial vasoconstric-
tion (in spite of a similar increase in MAP). Thus, the initial vasoconstriction caused by cigarette smoking seems likely to be due not to the airborne nicotine in cigarette smoke but to some smoking-induced substance. Since the vasoconstriction was blocked by seratrodast, the substance responsible for vasoconstriction is presumably TxA2. We also found that mecamylamine, propranolol, L-NAME, and glibenclamide all reduced or prevented the dilation of cerebral vessels induced by acute cigarette smoking in the present rat model. The mechanism by which inhalation of mainstream smoke from a low or high nicotine-containing cigarette causes the vascular tone to decrease could be activated by nicotine and involve, at least in part, sympathetic activation, stimulation of NO production, and the opening of ATP-sensitive K+ channels.

The effects of short-term smoking on the cardiovascular system have been reported to be mediated by an immediate release of catecholamines from local adrenergic terminals, followed by a systemic release from the adrenal medulla, as evidenced by the early rise primarily of norepinephrine, followed by a rise in epinephrine level. Nicotine is described as a ganglion-stimulating drug; in fact, it stimulates both sympathetic ganglia and the adrenal medulla. Mecamylamine has been reported to block neuronal nicotinic receptors in various locations, including in the brain, ganglia, and spinal cord, and to exhibit both competitive and noncompetitive properties in antagonizing the central effects of nicotine. A wide range of doses given by different routes (0.125 mg/kg PO to 5 mg/kg IV) have been found to be effective at antagonizing the effects of nicotine in previous rat studies. Since with an intravenous dose of 1.0 mg/kg mecamylamine it was difficult to keep MAP at 90 mm Hg or above, we used a dose of 0.7 mg/kg IV in the present study.

**Figure 2.** Line graphs showing the effects of mecamylamine (A), propranolol (B), L-NAME (C), and glibenclamide (D) on the responses to smoking a 1 mg nicotine-containing cigarette. The initial vasoconstriction observed was not affected by mecamylamine, propranolol, L-NAME, or glibenclamide. The smoking-induced vasodilation was completely inhibited by mecamylamine, greatly inhibited by glibenclamide, and reduced by propranolol or L-NAME. Values are mean±SEM (control, n=6; mecamylamine, n=6; propranolol, n=6; L-NAME, n=6; and glibenclamide, n=6). *P<0.05, †P<0.01 compared with baseline.

**TABLE 2. Changes in Blood Glucose Concentration During and After Smoking in the Presence of Antagonists**

<table>
<thead>
<tr>
<th>Glucose, mg/dL</th>
<th>Before Treatment</th>
<th>Baseline</th>
<th>1 min</th>
<th>5 min</th>
<th>15 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mecamylamine</td>
<td>158±16</td>
<td>145±5</td>
<td>143±10</td>
<td>149±12</td>
<td>153±13</td>
<td>165±9</td>
</tr>
<tr>
<td>Propranolol</td>
<td>128±9</td>
<td>137±15</td>
<td>154±27</td>
<td>149±26</td>
<td>161±16*</td>
<td>168±11†</td>
</tr>
<tr>
<td>L-NAME</td>
<td>132±8</td>
<td>129±19</td>
<td>131±18</td>
<td>133±15</td>
<td>137±18</td>
<td>135±16</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>139±8†</td>
<td>110±21</td>
<td>92±14*</td>
<td>86±9†</td>
<td>79±†</td>
<td>93±4*</td>
</tr>
<tr>
<td>Seratrodast</td>
<td>144±12</td>
<td>154±11</td>
<td>157±7</td>
<td>150±7</td>
<td>135±7</td>
<td>135±10</td>
</tr>
</tbody>
</table>

Values are mean±SEM.
*P<0.05, †P<0.01 compared with baseline.
The complete inhibition of the smoking-induced vasodilation by mecamylamine would demonstrate that the vasodilation is most likely initiated by nicotine via an activation of nicotinic receptors.

In canine experiments, nicotine seems to cause predominantly β-adrenoceptor–mediated vasodilation in the cerebral cortex, although it also activates α-adrenoceptors and a nonadrenergic, noncholinergic vasodilator mechanism. The present attenuation of the smoking-induced vasodilation by propranolol would indicate that the vasodilation is, at least in part, achieved via β-adrenergic stimulation. The significant increase in blood glucose concentration observed in the present study might be indirect evidence of smoking-induced sympathetic nerve stimulation. The transient increase in MAP without a change in HR at the end of smoke inhalation observed in the present study is presumably due to a smoking-induced sympathetic activation. Referring to the previous studies, we used 1 mg/kg IV propranolol for effective blocking of β-adrenoceptors. Although we cannot exclude the possibility that a complete inhibition with propranol could occur if we used a different timing or dosage, we suspect that the pial vessel response to smoking cannot be attributed entirely to sympathetic β-adrenoceptor stimulation.

Recent in vivo studies have suggested that ATP-sensitive K⁺ channels are present in cerebral arterioles. The opening of such channels in the vascular smooth muscle cell causes vasorelaxation. The complete inhibition of smoking-induced pial vasodilatation by glibenclamide, a putative ATP-sensitive K⁺ channel blocker, suggests that this response is probably induced via an activation of ATP-sensitive K⁺ channels in the cerebral arterioles. Glibenclamide has been shown to block vascular K⁺ channels directly and to competitively antagonize vasodilatation evoked by ATP-sensitive K⁺ channel openers such as diazoxide and cromakalim. Indeed, we have previously demonstrated that topical application of glibenclamide can effectively block cromakalim-induced pial arteriolar and venular dilation in the dog in vivo. In rat studies, the following occurred: (1) 20 mg/kg of glibenclamide given intravenously blunted the vasodilator effect of the K⁺ channel opener, diazoxide, but not that of the L-type Ca²⁺ channel blocker, nicardipine, and (2) 20 to 30 mg/kg of glibenclamide given intravenously inhibited the vasorelaxant

### TABLE 3. MAP and HR Changes During Experiments With Antagonists

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>MAP, mm Hg</th>
<th>HR, bpm</th>
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<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mecamylamine</td>
<td>123±7*</td>
<td>388±14</td>
</tr>
<tr>
<td>Propranolol</td>
<td>132±4</td>
<td>405±8</td>
</tr>
<tr>
<td>L-NAME</td>
<td>125±5*</td>
<td>400±9</td>
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<td>Glibenclamide</td>
<td>121±8</td>
<td>394±7</td>
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<tr>
<td>Seratrodast</td>
<td>118±5</td>
<td>394±10</td>
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</table>

Values are mean±SEM. *P<0.05, †P<0.01 compared with baseline.

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**Figure 3.** Line graph showing the effect of seratrodast on the response to smoking a 1 mg nicotine–containing cigarette. The initial vasoconstriction was completely inhibited by seratrodast, and the subsequent vasodilatation was prolonged (it persisted for at least 60 minutes). Groups are as follows: control (n=6) and seratrodast (n=6). *P<0.05, †P<0.01 compared with baseline.

**Figure 4.** Plasma TxB₂ concentration was significantly elevated after smoking; after nicotine infusion, it was not changed. Values are mean±SEM (smoking, n=6; nicotine infusion, n=6). *P<0.01 compared with baseline.
The effects of cromakalim and diazoxide in vivo. The dose of glibenclamide used in the present study was similar to the doses used on previous studies. Since a recent in vivo study suggested that ATP-sensitive K\textsubscript{ATP} channels play an important role in metabolic coronary vasodilation associated with \( \beta \)-adrenoceptor activation, glibenclamide may have modulated the effects of sympathetic activation induced by cigarette smoking.

In addition, since NO acts as a neurotransmitter in the vasodilator nerves innervating the cerebral arterial wall, we anticipated that NO might also play a role in smoking-induced pial vasodilation. In fact, the release of acetylcholine induces NO production by the endothelial cells. In practice, the smoking-induced pial vasodilation was only partially attenuated by L-NAME pretreatment, and its duration was actually prolonged (from 30 to 60 minutes). Systemic administration of L-NAME (3 to 190 mg/kg IV) is widely used in physiological experiments in vivo to produce NO synthase inhibition. In a recent report, intravenous administration of L-NAME partially inhibited the catalytic activity of brain NO synthase (assayed ex vivo) in a time- and dose-dependent fashion in the rat. Thus, 5 or 10 to 40 mg/kg of L-NAME administered intravenously resulted in NO synthase inhibition by 26% and 40%, respectively, 30 minutes after a bolus injection, and 20 mg/kg of L-NAME resulted in a stable 52% inhibition within 2 hours. The continuous intravenous infusion of L-NAME at 10 mg \( \cdot \) kg\textsuperscript{-1} \( \cdot \) h\textsuperscript{-1} used in the present study might not have been enough to achieve complete inhibition of NO synthase, but the fact that the systemic hypertension occurred after the infusion seems to suggest that vascular endothelial NO synthase was substantially inhibited. Furthermore, it is possible that the systemic effects associated with L-NAME administration (a decrease in pH) might have been responsible for such a sustained dilation of pial arterioles.

The fact that attenuated responses are associated with repeated cigarette smoke inhalation has been reported by others. We found evidence that short-term repeated smoking of 1 mg nicotine–containing cigarettes attenuated the pial vasodilation. Since the MAP change was not affected by such

**Figure 5.** The initial vasoconstriction observed after smoking was still present after repeated smoking, but the smoking-induced vasodilation was attenuated. Values are mean±SEM (single, \( n=6 \); repeated, \( n=6 \)). *\( P<0.05 \), †\( P<0.01 \) compared with baseline.

**TABLE 4.** Changes in HbCO and Blood Glucose Concentration During and After Single or Repeated Cigarette Smoking

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>1 min</th>
<th>5 min</th>
<th>15 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbCO, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>2.9±0.6</td>
<td>7.8±0.9†</td>
<td>6.8±0.8†</td>
<td>6.7±0.6†</td>
<td>5.7±0.5*</td>
</tr>
<tr>
<td>Repeated</td>
<td>6.4±0.6</td>
<td>8.3±0.5*</td>
<td>9.0±0.9†</td>
<td>7.4±0.6</td>
<td>5.5±0.3</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>145±6</td>
<td>152±9</td>
<td>161±5</td>
<td>171±14†</td>
<td>155±5</td>
</tr>
<tr>
<td>Repeated</td>
<td>141±11</td>
<td>137±11</td>
<td>140±11</td>
<td>134±14</td>
<td>140±14</td>
</tr>
</tbody>
</table>

Values are mean±SEM. *\( P<0.05 \), †\( P<0.01 \) compared with baseline.
repeated smoking, a rapid adaptation of the response of the pial arterioles might have occurred, perhaps because of central effects on the sympathetic innervation caused by repeated smoking. In addition, it has been demonstrated that endothelium-dependent vasodilation of the brachial arteries is impaired in apparently healthy young adult smokers and that long-term smoking is associated with a diminution of the NO-dependent component of basal vascular tone. Such effects could account for our finding that repeated smoking (even in the short term) causes an impairment of cerebral vasodilation; in that case, the cause would be endothelial dysfunction. A significant increase of the plasma concentration of HbCO after both single and repeated cigarette smoking could also affect the vascular responses. Traystman and colleagues have reported that elevated levels of HbCO produce a leftward shift of the oxyhemoglobin dissociation curve for the remaining O₂-available binding sites and that this is the factor responsible for the excessive CBF response. It is possible that a mechanisms of the vasodilator response observed after single cigarette smoking may be due to elevated levels of HbCO. Nevertheless, baseline high concentration of HbCO and repeated exposure to CO may attenuate the vasodilator response associated with smoking. One more difference between single and repeated cigarette smoking on the cerebral vasculature was glucose concentration. It has been reported that acute hyperglycemia decreases cerebrovascular resistance and increases CBF; thus, hyperglycemia after single-cigarette smoking may modulate the vasodilator effect. The initial vasoconstriction remained in the repeated inhalation experiment, so if we can extrapolate from these results, the slight but significant initial vasoconstriction may be present and have some physiological importance in the chronic smoker.

Previous studies have demonstrated CBF changes at only 1 or 2 time points during smoking, and in no study has the time course of the cerebrovascular responses been examined during and after smoking. The initial constriction of pial vessels during smoking might not be entirely due to the pharmacological action of cigarette smoke. However, some vasconstrictors—such as airway smooth muscle TxA₂ in guinea pigs, plasma endothelin-1 in humans, and cerebral cortex serotonin in anesthetized rats—have been found to be elevated after smoking or nicotine administration. In the present study, the arterial TxB₂ level, a product of TxA₂, was found to be significantly elevated early on in the period under study, and a TxA₂ receptor antagonist, seratrodast, inhibited the initial pial vasoconstriction. Seratrodast has been reported to competitively inhibit contractions of guinea pig tracheal strips and saphenous vein strips in response to the TxA₂ mimic, U-46619, but it has not been reported to inhibit the contractions of tracheal strips induced by leukotriene D₄, platelet-activating factor, or histamine. Seratrodast also competitively inhibits the binding of [³H]U-46619 to Chinese hamster ovary cells into which the TxA₂ receptor—coding gene has been introduced and which stably express the human TxA₂ receptor. These findings suggest that the pharmacological effects of seratrodast are due to its antagonism of TxA₂ receptors. Since seratrodast at doses of 0.08 to 5 mg/kg suppressed the bronchoconstriction caused by intravenous U-46619 in an in vivo study, the intravenous dose of 5 mg/kg seratrodast used in the present study should block or at least reduce a TxA₂-induced vasoconstriction. Therefore, at least in part, smoking-induced TxA₂, which can cross the blood-brain barrier, would be able to cause the initial pial vasoconstriction. Although we did not measure circulating endothelin and serotonin concentrations (because they do not cross the blood-brain barrier), we cannot exclude the possibility that endothelin or serotonin locally induced by smoking might also cause pial vessels to constrict.

In the present study, there might be several limitations that should be considered in evaluating the cerebrovascular changes related with smoking inhalation. The increase of MAP from a minimum of 97 to 109 mm Hg to a maximum of 126 to 163 mm Hg that was observed for 1 minute during cigarette smoking should affect the autoregulatory response in cerebral vessels. In the steady-state responses to hypertension in a feline study, smaller (37 to 59 μm in diameter) and larger (117 to 174 μm in diameter) vessels did not show any significant change in caliber until blood pressure was elevated to 170 and 190 mm Hg, respectively. Considering the difference in size between rats and cats, it is uncertain whether pial arterioles in rats (21 to 75 μm) would respond similarly to the same-sized vessels in cats. In a previous rat study, cortical arterioles with a resting diameter of 20 to 70 μm responded by nearly equal proportional changes in diameter over 65 to 155 mm Hg of systemic MAP for constant CBF. Therefore, the early phase of the response until MAP became stable during and after cigarette smoking or nicotine infusion may be partially modulated by the autoregulatory response in cerebral vessels. And pretreatment with mecamylamine or L-NAME caused baseline MAP decrease or increase. Although an increase in MAP associated with L-NAME did not change the baseline diameter of pial arterioles, an 8.2% increase in the arteriolar diameter associated with mecamylamine would be possible to partially change the pial arteriolar response to cigarette smoke per se. Since the effects of mainstream cigarette smoke on the venules would be mostly passive when cardiovascular changes occur, it would not be reasonable to consider the possible mechanism of such an effect on the pial venule as a result of cigarette smoking. Perhaps the vasodilator stimuli can dilate vessels upstream, resulting in an increase in downstream capillary and venular microvascular pressure. Venular changes could appear to be the summation of the direct and indirect (passive) influences of smoking. Thus, we only showed the effect of cigarette smoking on cerebral pial venules.

Although we have clearly demonstrated that acute cigarette smoking causes an initial constriction and a subsequent dilation of pial arterioles and although we have provided evidence to help identify some of the mechanisms involved, it would be speculative to extrapolate from our results to humans, if only because of the species differences. However, the plasma nicotine concentrations after 1 mg nicotine-containing cigarette smoking measured in the present study (35.0 ± 9.3 ng/mL) were quite consistent with those recorded in human studies after single-cigarette smoking (33 ng/mL on average) in 330 smokers, greater than 60 ng/mL when a
cigarette was “smoked deeply,”51 and 15±3 ng/mL in 12 smokers.52). At least on this basis, the results of the present study may be relevant to the human smokers.

In summary, cigarette smoking appears to have a significant biphasic effect on tone in the cerebral vasculature. Nicotine itself induced vasodilation of pial vessels in a dose-dependent manner. The mechanisms underlying the vasodilation induced by cigarette smoke may be initiated by nicotine by means of an activation of nicotinic receptors. This may cause vasodilation, at least in part, by sympathetic activation, NO production, and K+ channel activation. The initial vasoconstriction seems to be caused by the other constituents of cigarette smoke, which may induce TXA2. In addition, the pial vasodilation caused by smoking shows attenuation on repeated smoking. Thus, multiple mechanisms could be involved in the response of pial arterioles to cigarette smoking.

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References

In this interesting article, the authors attempt to determine the mechanisms underlying the cerebrovascular effects of cigarette smoking in rats in vivo. The authors used a standard pial window technique to examine the effects of cigarette smoke and nicotine and a variety of blocking agents to determine the potential mechanisms of nicotine’s vasoconstrictory and vasodilatory effects on the pial vessels. The authors found that cigarette smoking had a biphasic effect on cerebrovascular tone, i.e., vasoconstriction followed by vasodilation. The authors used a variety of pharmacological agents to demonstrate that the vasodilation is most likely an effect of nicotine, at least in part mediated via sympathetic activation, nitric oxide production, and K⁺ channel activation. They speculate that the vasoconstriction is due partly to thromboxane A₂ induced by cigarette smoke.

The effect of cigarette smoke on human physiology and pathophysiology has been a hotly debated issue for many decades, and while most researchers believe that cigarette smoke in many ways is harmful to physiological systems, absolute hard data have not been easy to come by. In this study, the effects of cigarette smoke to result in cerebral vasoconstriction followed by vasodilation seems clear. The critical question, however, is this: What are the mechanisms for these responses? One of the problems centers around the fact that cigarette smoke contains a multitude of constituents, and thus the effects of cigarette smoke on the cerebrovasculature may be a net effect of not only nicotine but many, many other constituents in mainstream cigarette smoke as well. In mainstream cigarette smoke, carbon monoxide, acetaldehyde, nitric oxides, hydrogen cyanide, acrolan, ammonia, tobacco alkaloids, pyrites, and particulate matter are but only a few of cigarette smoke’s constituents. Precisely which of these agents have direct effects on the cerebrovasculature, or which of these agents affect or modulate other responses, is completely unclear. There may be additive and/or synergistic effects of all of these agents. Thus, the absolute mechanisms are unclear. There are also issues related to acute cigarette smoke, chronic cigarette smoke, and secondary cigarette smoke, and how the constituents within cigarette smoke may affect the cerebrovasculature differently under all of these conditions. Certainly, cigarette smoking for 20 years at a high volume may have completely different effects than cigarette smoking of only a few acute cigarettes. These issues need to be considered when examining the effects of cigarette smoke on physiological and pathophysiological parameters. Merely because a specific agent such as nicotine causes an effect that appears similar to the effect of cigarette smoking does not necessarily mean that nicotine is the major culprit in altering the physiological response. Coupled with all of the other agents in cigarette smoke, one does not know the other modulating factors. There may be an association, but it may not in fact be a cause-and-effect relationship. Similarly, one of the major constituents of cigarette smoke is carbon monoxide. When inhaled, the combination with hemoglobin produces elevated levels of carboxyhemoglobin and results in hypoxia. Under conditions of hypoxia, the effects of the other constituents of cigarette smoke may be modified by virtue of the hypoxic response of the cerebral vessels. Thus, the issues related to the effects of cigarette smoke and the cerebrovasculature again remain unclear despite the fact that it is almost a certainty that constituents in cigarette smoke can modify the cerebrovasculature itself. And as shown in this manuscript, nicotine, as one constituent of cigarette smoke, clearly does alter cerebral blood vessels under these methodological circumstances.

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Mechanisms Underlying Cerebrovascular Effects of Cigarette Smoking in Rats In Vivo
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