Parasympathetic Coronary Vasoconstriction Induced by Nicotine in Conscious Calves

Mark A. Young, Delvin R. Knight, and Stephen F. Vatner

We studied the effects of intracoronary injection of nicotine and acetylcholine on coronary blood flow in nine conscious calves chronically instrumented to measure coronary blood flow, left ventricular (LV) and mean arterial pressure, LV dP/dt, and heart rate. Nicotine (5 μg/kg i.c.) elicited a biphasic response in coronary blood flow consisting of an initial vasoconstriction (phase 1; blood flow fell by 52 ± 5.4% from a baseline of 66 ± 7.5 ml/min) followed by vasodilation (phase 2; blood flow rose 119 ± 12.7% above baseline). The change in coronary blood flow with nicotine was not associated with changes in LV systolic pressure, mean arterial pressure, or heart rate. The change in coronary blood flow was unaffected by combined α- and β-adrenoceptor blockade with prazosin, rauwolscine, and propranolol but was abolished by either muscarinic blockade with atropine or ganglionic blockade with hexamethonium. Acetylcholine (0.5 μg/kg i.c.), without affecting mean arterial pressure, elicited changes in coronary blood flow similar to those observed with nicotine, producing an initial phase of coronary vasoconstriction (blood flow fell by 71 ± 4.9%) followed by vasodilation (blood flow rose by 228 ± 20.7%). Both phases of the response to acetylcholine were abolished by muscarinic blockade but were unaffected by ganglionic blockade. When nicotine was injected into the left circumflex coronary artery, no change in blood flow was observed in the left anterior descending coronary artery, indicating the lack of involvement of global reflex pathways. These results suggest that nicotine locally stimulates parasympathetic nerves, which constrict the coronary circulation via a muscarinic mechanism. (Circulation Research 1988; 62:891-895)

Studies of vascular tension in vitro have traditionally allowed muscarinic agonists to preconstrict coronary vessels. However, muscarinic contraction was not consistent with the observation that administration of acetylcholine in vivo elicited only profound increases in coronary blood flow. The apparent inconsistency was partially resolved by the widely recognized observations of Furchgott, who demonstrated the necessity of intact endothelial cells in the production of muscarinic vasodilation. Yet other studies have implied that even with intact endothelium, muscarinic stimulation with exogenous agents causes coronary vasoconstriction in both human and nonhuman primates as well as in bovine and porcine models.

Nicotine has diverse cardiovascular actions, including stimulation of mechanoreceptors and chemoreceptors, depolarization of postganglionic neurons, and release of the respective neurotransmitters. Studies have demonstrated that the vascular actions of nicotine are due to both sympathetic and parasympathetic stimulation of vascular smooth muscle. Priola et al have demonstrated that intracoronary nicotine stimulates the release of acetylcholine from intrinsic cardiac nerves. We, therefore, chose to examine the effects of intracoronary injection of nicotine and acetylcholine on the control of coronary blood flow in conscious calves. Intracoronary injection obviates the use of high doses of nicotine frequently reported in other studies, and the study of conscious animals avoids the neural and vascular depressant effects of recent surgery and anesthesia.

Materials and Methods

The effects of nicotine (5 μg/kg i.c. bolus) and acetylcholine (0.5 μg/kg i.c. bolus) in nine conscious calves instrumented 1-3 weeks previously were studied. Left ventricular (LV) and aortic pressure, LV dP/dt, left circumflex coronary blood flow, and heart rate were measured. The details of this model have been published previously. Using sterile surgical technique, a Doppler ultrasonic blood flow transducer and hydraulic occluder were implanted around the left circumflex coronary artery. Proximal to the Doppler flow transducer, a Silastic catheter was implanted in the circumflex coronary artery using the method of Herd and Barger. A Tygon catheter was implanted in the descending aorta. In five animals, the left anterior descending coronary artery was also instrumented with an intracoronary catheter and Doppler flow transducer. The catheters and lead wires were externalized between the ribs to the interscapular space. The incision was closed, the chest evacuated, and the animals allowed to recover. Catheters were flushed daily with heparin, and the animals were given 6,000,000 units of penicillin on alternate days for 10 days. Animals used in this study were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School.
School and the National Institutes of Health’s “Guide for Care and Use of Laboratory Animals.”

Statistical significance was tested using a one-way analysis of variance and subsequent comparison of means using Duncan’s multiple range test (SPSS statistics package). All data are reported as mean ± SEM. Statistical significance was accepted at p<0.05.

On the day of the experiment, nicotine (5 µg/kg) and acetylcholine (0.5 µg/kg) were injected as an intracoronary bolus. Repeated injections of either agent showed no evidence of tachyphylaxis. To determine the mechanism of action of nicotine and acetylcholine in the coronary circulation, the administration of each agonist was repeated on separate days in the presence of muscarinic receptor blockade with atropine (0.1 mg/kg i.v.) or ganglionic blockade with hexamethonium (10 mg/kg i.v.). In addition, nicotine was administered in the presence of β-adrenoceptor blockade with propranolol (1 mg/kg i.v.) or combined β-, α₁- and α₂-adrenoceptor blockade with propranolol, prazosin (0.01 mg/kg i.c.), and rauwolscine (0.005 mg/kg i.c.).

### Table 1. Effects of Nicotine (5 µg/kg) Injected in Left Circumflex Coronary Artery of Nine Intact Conscious Calves

<table>
<thead>
<tr>
<th>Phase</th>
<th>LCCA blood flow (ml/min)</th>
<th>Percent change from control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>66 ± 7.5</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Phase 1</td>
<td>-52 ± 5.4 *</td>
<td>119 ± 12.7 *</td>
</tr>
<tr>
<td>Phase 2</td>
<td>0 ± 2.0 t</td>
<td>8 ± 4.3 t</td>
</tr>
<tr>
<td>α+β-blockade</td>
<td>95 ± 5.3</td>
<td>-58 ± 3.7 *</td>
</tr>
<tr>
<td>Muscarinic blockade</td>
<td>67 ± 5.0</td>
<td>-6 ± 5.0 t</td>
</tr>
<tr>
<td>Ganglionic blockade</td>
<td>57 ± 7.5</td>
<td>0 ± 2.0 t</td>
</tr>
</tbody>
</table>

### Table 2. Effects of Acetylcholine (0.5 µg/kg) Injected in Left Circumflex Coronary Artery of Eight Intact Conscious Calves

<table>
<thead>
<tr>
<th>Phase</th>
<th>LCCA blood flow (ml/min)</th>
<th>Percent change from control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>85 ± 4.4</td>
<td>-71 ± 4.9 *</td>
</tr>
<tr>
<td>Phase 1</td>
<td>228 ± 20.7 *</td>
<td></td>
</tr>
<tr>
<td>Phase 2</td>
<td>15 ± 5.4 t</td>
<td></td>
</tr>
<tr>
<td>Muscarinic blockade</td>
<td>89 ± 1.7</td>
<td>-1 ± 2.6 t</td>
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<tr>
<td>Ganglionic blockade</td>
<td>77 ± 4.3</td>
<td>-80 ± 4.0 t</td>
</tr>
</tbody>
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The coronary and cardiac effects of nicotine may arise from activation of either or both mechanoreceptors and chemoreceptors and resultant reflex neural activation. To test the possibility that global activation of cardiac neural pathways could account for the coronary effects of nicotine, in five animals the blood flow in the left anterior descending coronary artery during injection of nicotine into the left circumflex coronary artery was measured.

### Results

In the control state, with the awake animal lying quietly in a cart, baseline circumflex coronary blood flow averaged 66 ± 7.5 ml/min. Intracoronary nicotine elicited a biphasic response in coronary blood flow (Figure 1) consisting of a profound fall in coronary blood flow (−52 ± 5.4%, phase 1) followed by a vasodilation (+119 ± 12.7%, phase 2). This coronary response was accompanied by insignificant changes in LV systolic pressure, mean arterial pressure, and heart rate and by a small rise in LV dp/dt (Table 1). Neither the coronary constriction (phase 1) nor the dilation (phase 2) was affected by β- or α-adrenoceptor blockade (Table 1). In marked contrast, both phases of the coronary response to nicotine were abolished following either muscarinic blockade with atropine or ganglionic blockade with hexamethonium.

In eight animals, acetylcholine produced a biphasic change in left circumflex coronary blood flow that was nearly identical to that observed with nicotine (Table 2). Coronary blood flow was transiently reduced by 71 ± 4.9% from a baseline of 85 ± 4.4 ml/min in the absence of significant changes in arterial pressure and heart rate and subsequently rose by 228 ± 20.7% above control. As expected, both phases of the response to acetylcholine were abolished by muscarinic blockade with atropine. The coronary response to acetylcholine was not affected by ganglionic blockade with hexamethonium (Figure 1, Table 2).

In five animals, left anterior descending coronary blood flow was measured during injection of nicotine into the left circumflex coronary artery. At the time of peak changes in left circumflex coronary blood flow, left anterior descending coronary blood flow did not change during phase 1 or phase 2. In two animals, injection of nicotine into the left anterior descending coronary artery resulted in responses similar to those described above for nicotine injected into the left
Intact Ganglionic Blockade

LV Pressure (mm Hg)
LV dP/dt (mm Hg/sec)
Arterial Pressure (mm Hg)
Mean Arterial Pressure (mm Hg)
Mean LCCA Blood Flow (ml/min)
Heart Rate (bts/min)

FIGURE 1. Effects of intracoronary nicotine (5 μg/kg) on left ventricular (LV) pressure, LV dP/dt, phasic and mean arterial pressure, phasic and mean left circumflex coronary blood flow, and heart rate in an intact conscious calf (left panel). Right panels: Responses to nicotine (5 μg/kg) (with spontaneous heart rate) and acetylcholine (0.5 μg/kg) (with heart rate paced above normal rate to avoid bradycardia) in the same animal following ganglionic blockade with hexamethonium. Hexamethonium abolished both the coronary blood flow response and inotropic response to nicotine, suggesting involvement of autonomic nerves. Coronary response to acetylcholine was unchanged following hexamethonium demonstrating that vascular reactivity was not depressed.
circumflex artery (i.e., left anterior descending coronary blood flow fell by 45% during phase 1 and rose by 169% during phase 2).

**Discussion**

Nicotine has been widely used as a pharmacological tool to examine cigarette smoking-related vascular reactivity as well as to study the associated reflex coronary vascular changes. Most studies have emphasized that the direct (nonreflex) actions of nicotine on vascular smooth muscle are mediated via α- and β-adrenergic mechanisms, although muscarinic mechanisms have also been implicated. The present study demonstrates that nicotine elicits coronary vasoconstriction via a muscarinic mechanism with little involvement of neurally released or circulating catecholamines.

The major new finding of the present study is the demonstration of striking muscarinic vasoconstriction induced by nicotine, potentially mediated via the parasympathetic nerve terminals. This is in contrast to what has previously been reported in the coronary circulation with intravenous or intracarotid administration of nicotine or with inhaled cigarette smoke. The present results with intracoronary nicotine and acetylcholine also differ from the widely recognized acetylcholine-induced vasodilation previously reported in the canine coronary circulation. However, muscarinic vasoconstriction with exogenous agents has been demonstrated in porcine, bovine, and nonhuman primate models of the coronary circulation, and recent reports of muscarinic vasoconstriction in humans with coronary artery disease suggest that parasympathetic mechanisms may be implicated in the pathogenesis of coronary vasospasm. It is of interest from this standpoint that the dose of nicotine used in the present study is similar to the dose calculated by Folts and Bonebrake to equal that obtained from smoking a single cigarette.

In the present study, the effects of nicotine were abolished by the nicotinic receptor blocker hexamethonium as well as by the muscarinic antagonist atropine. This, taken together with the lack of effect of hexamethonium on acetylcholine-induced coronary constriction, suggests that nicotine acts on the cardiac parasympathetic nerves to release acetylcholine, which in turn constricts the coronary circulation. The data further suggest that the vasoconstriction is not reflexly mediated because injection of nicotine was not accompanied by concurrent coronary flow changes in the unexposed (contralateral) coronary bed. Instead, nicotine most likely stimulates cholinergic nerve terminals through a local activation of nicotinic receptors. Whether nicotine stimulates parasympathetic nerve endings, axons, or intracardiac ganglia cannot be discerned from these data. That nicotine stimulates the release of acetylcholine from parasympathetic nerves is supported by the similarity of the coronary response to nicotine and acetylcholine. It has also been demonstrated by Priola et al and Blomquist et al that intracoronary nicotine elicits negative dromotropic effects in the arterioventricular node of cardiac denervated dogs and negative inotropic effects in the ventricle of innervated, β-blocked dogs. These reports conclude that postganglionic parasympathetic neurons originating in the atria mediate the negative inotropic and dromotropic effects of intracoronary nicotine. In this connection, the present results support the work of Priola et al and Blomquist et al. However, the response of coronary blood flow to nicotine was not examined in those studies. Moreover, previous studies in the canine coronary circulation suggest only vasodilation in response to parasympathetic stimulation. Thus, the qualitative difference in the calf (i.e., potent coronary vasoconstriction) from what would have been predicted from prior canine experiments further points out the importance of examining species alternative to the dog for a potentially better understanding of the human coronary circulation.

In conclusion, the present study, nicotine slightly elevated heart rate and LV dp/dt. This is most likely a local effect on cardiac adrenergic nerve terminals because intravenous administration of nicotine (5 μg/kg), which would be expected to reach extracardiac sympathetic ganglia, is without effect on coronary blood flow, heart rate, or LV dp/dt. The blockade of nicotine's actions by hexamethonium most likely occurs at nicotinic receptors on both parasympathetic and sympathetic nerves because both the muscarinic-mediated changes in coronary blood flow and the adrenergically mediated increases in LV dp/dt and heart rate seen with nicotine are abolished by hexamethonium (Table 1). The small changes in coronary blood flow that persist in the presence of muscarinic blockade (Table 1) reflect the adrenergically mediated component of nicotine administration. These adrenergic effects are minimal, however, because the response to nicotine was not significantly affected by combined α- and β-adrenoceptor blockade.

The nature of the biphasic response to nicotine is unclear. The dilation that follows the initial period of vasoconstriction with nicotine is most likely muscarinic because it is abolished by atropine but unaffected by adrenergic blockade. Potentially a portion of the coronary dilation (phase 2) in response to either nicotine or acetylcholine is due to reactive hyperemia following the flow debt incurred during the vasoconstriction (phase 1). Whether this biphasic response (constriction followed by dilation) is also a phenomenon dependent on temporal changes in local vascular concentration of acetylcholine or due potentially to activation of two distinct receptor subtype populations cannot be answered by the present study.

In conclusion, the present study in conscious calves demonstrates that intracoronary nicotine elicits potent coronary vasoconstriction followed by vasodilation. This biphasic response is most likely mediated by acetylcholine, which is released by a direct action of nicotine on the parasympathetic nerves. These results, in addition to elucidating a potentially new pathway of coronary vasoconstriction, could be important in even-
tually understanding mechanisms mediating coronary vasospasm.

References


Key Words: nicotine • coronary blood flow • coronary vasoconstriction • acetylcholine
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