Nicotine Promotes Arteriogenesis

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OBJECTIVES In the current study, we used a model of limb ischemia to determine whether nicotine could enhance arteriogenesis, to compare the magnitude of this effect to the angiogenic factor basic fibroblast growth factor (bFGF), and to investigate the mechanisms of the effect.

BACKGROUND We have shown previously that nicotine stimulates angiogenesis via stimulation of endothelial nicotinic cholinergic receptors. Stimulation of endothelial nicotinic cholinergic receptors causes endothelial cell proliferation, migration, and formation of capillary networks in vitro and angiogenesis in vivo in conditions of ischemia and inflammation.

METHODS New Zealand White rabbits (n = 85) underwent unilateral femoral artery occlusion and were randomized to nicotine (0.05 to 5.0 μg/kg/day), bFGF (10 μg/kg/day), or vehicle delivered intra-arterially via osmotic minipumps. At day 21, morphologic changes were assessed by immunohistochemistry and angiography. Blood flow in the ischemic limb was determined by intra-arterial Doppler flow measurements and microsphere distribution.

RESULTS Nicotine enhanced capillary density in the ischemic hind-limb to a similar extent as bFGF. Nicotine also increased angiographic score, calf blood pressure ratio, intra-arterial Doppler flow, and microsphere distribution. In vitro, nicotine stimulated monocyte adhesion and transmigration. Nicotine increased by two- to three-fold the expression of monocyte adhesion molecules CD11b and CD11a; the expression of the endothelial adhesion molecule intercellular adhesion molecule-1; and the endothelial release of monocyte chemoattractant protein-1.

CONCLUSIONS In the short term, nicotine promotes angiogenesis and arteriogenesis in the setting of ischemia. The effect of nicotine may be mediated in part by activation of endothelial-monocyte interactions involved in arteriogenesis. (J Am Coll Cardiol 2003;41:489–96)

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Nicotinic acetylcholine receptors (nAChRs) are expressed in neuronal, as well as non-neuronal cells such as endothelial cells (ECs) and vascular smooth muscle cells. In non-neuronal cells these receptors may be involved in the regulation of mitosis, differentiation, organization of the cytoskeleton, cell-cell contact, locomotion, and migration (1–3). Acetylcholine is synthesized and stored in ECs, suggesting that acetylcholine may act as an autocrine factor in the cardiovascular system (3,4).

We recently demonstrated that nicotine stimulates angiogenesis in a variety of models via stimulation of nAChRs (5). Nicotine increased EC number, reduced apoptosis, and increased capillary network formation in vitro as well as enhanced angiogenesis/neovascularization in murine models of inflammation, tumor, atherosclerotic plaque, and ischemia. The acceleration of pathologic angiogenesis increased the growth of tumors or atheromas. The enhancement of therapeutic angiogenesis in the hind-limb ischemia model increased limb blood flow as assessed by magnetic resonance perfusion imaging.

However, a deficiency of the murine models is that it is difficult to document the impact of nicotine on arteriogenesis. There are salient differences between angiogenesis and arteriogenesis. Angiogenesis or capillary sprouting results when an angiogenic stimulus induces ECs in the existing vasculature to proliferate and migrate through the tissue to form new endothelialized channels, typically capillaries or small arterioles (6). In contrast, arteriogenesis is a result of growth and positive remodeling of pre-existing collaterals into larger conduits that can be visualized angiographically (7,8). There is evidence that monocytes are involved in arteriogenesis. Arras et al. (9) has shown that monocyte chemoattractant protein-1 (MCP-1) enhances collateral formation in a rabbit model of hind-limb ischemia. Both angiogenesis and arteriogenesis processes occur in response to ischemia but their relative contribution to the revascularization of ischemic tissue is not clear (10).

In this investigation, we provide the first evidence that nicotine can enhance arteriogenesis and reduce the hemodynamic defect in a rabbit model of critical limb ischemia. Nicotine is equipotent by comparison with basic fibroblast growth factor (bFGF). Furthermore, the effect of nicotine may be mediated by increased endothelial-monocyte interaction.

METHODS

Rabbit model of hind-limb ischemia. To investigate the effect of nicotine on angiogenesis and arteriogenesis we used...
a rabbit model of hind-limb ischemia (11). In New Zealand White rabbits (4.0 to 4.5 kg, male, n = 85; Pine Acre Rabbitry, Norton, Massachusetts), we ligated the femoral artery and its side branches (inferior epigastric, deep femoral, lateral circumflex, and superficial epigastric arteries). In this model of hind-limb ischemia, collateral artery development in the ischemic hind-limb model originates only from the internal iliac artery (11). A sterile polyethylene catheter is directed retrograde into the external iliac artery, with the tip of the catheter positioned in the common iliac artery proximal to the ostium of the internal iliac artery. The catheter itself is connected to a subcutaneously placed osmotic minipump (2ML-2, Durect Corp., Cupertino, California) (9). This pump is designed for continuous drug delivery over a period of two weeks. However, in preliminary experiments we determined that drug release continues until day 18. This pump permitted us to infuse nicotine for the majority of the postoperative period, with a drug-free period of three days before assessment of hind-limb perfusion. Rabbits were randomized for infusion of phosphate-buffered saline (PBS), bFGF (10 µg/kg/day), or nicotine (0.05, 0.1, 0.5, and 5.0 µg/kg/day; nicotine-free base; Sigma, St. Louis, Missouri) from day 1 to day 18. A single observer blinded to the treatment regimen performed all measurements.

Study end points. QUANTITATIVE ANGIOGRAM. Selective angiograms of the internal iliac artery were performed on day 0 and day 21. The infusion catheter was positioned in the internal iliac artery. After intra-arterial injection of adenosine (1 mg/min; Fujisawa USA, Deerfield, Illinois), a total of 5 ml of contrast media (Hypaque-76; Nycomed, Princeton, New Jersey) was injected using an automated injector (Medrad Mark IV, Pittsburgh, Pennsylvania) at a rate of 1 ml/s, and serial angiograms recorded. Quantitative angiographic analysis of the internal iliac artery was performed on a 10-mm long segment, 5 mm distal to the Doppler wire, on images recorded 4 s after injection of contrast. Collateral vessels were quantified with a grid overlay composed of circles 2.5 mm in diameter that were arranged in rows spaced 5 mm apart. The total number of grid intersections in the medial thigh area, as well as the total number of intersections crossed by a contrast-opacified artery, was counted. The angiographic score was the ratio of grid intersections crossed by contrast-opacified arteries divided by the total number of grid intersections (12).

HISTOLOGIC ASSESSMENT OF VESSEL FORMATION. Capillary density was determined in the adductor and semimembranous muscles. In 10-µm cryostat sections cut transverse to the midbelly of the muscle, capillaries were identified by monoclonal antibodies against CD146 (Chemicon, Temecula, California) (13). For each section, the capillary density was determined in 10 randomly selected fields using confocal microscopy and expressed as a ratio of capillaries to myocytes. Collateral arteries were defined as vessels with a total vessel diameter >100 µm and positive for both CD146 and smooth muscle alpha-actin.

VASOMOTOR REACTIVITY AND VASCULAR RESISTANCE. A 4F two-hole infusion catheter (Target Therapeutics, Freemont, California) was inserted into the right common carotid artery and advanced to the origin of the common iliac artery. A 0.018-inch Doppler guidewire (Cardiometrics, Mountain View, California) was advanced through this infusion catheter to the proximal segment of the internal iliac artery of the ischemic and normal limb, respectively. Resting average peak velocity (APV) was recorded after the velocity values had stabilized for at least 2 min. Maximum APV was recorded after bolus injection of 1 mg of adenosine (Sigma) in 0.2 ml of saline via the infusion catheter. The local adenosine infusion had no effect on systemic blood pressure. Doppler-derived flow (QD) was calculated as: \( Q_D = \frac{\pi d^2}{4} \times \frac{0.5 \times APV}{d} \), where \( d \) is vessel diameter. The mean velocity was estimated as 0.5 × APV by assuming a time-averaged parabolic velocity profile across the vessel. We used the diameter measurements from the angiogram recorded immediately before the Doppler measurements for the calculation of both rest and maximum flow. Flow reserve was calculated as the ratio of APV after adenosine injection divided by APV at baseline.

REGIONAL BLOOD FLOW TO LIMB MUSCLES. Regional perfusion of the ischemic hind-limb muscles was determined with E-Z TRAC Colored Microspheres (15 µm, Interactive Medical Technologies, Irvine, California). An infusion catheter was placed in the distal abdominal aorta for continuous infusion of adenosine (1 mg/min; 1 min before microsphere injection until 2 min after completion of microsphere injection). A second infusion catheter was placed in the left ventricle to allow intraventricular injection of colored microspheres (3 × 10^6 in 3 ml of saline; vortexcied and ultrasonicated). Tissue samples (8 g) of the quadriceps, semimembranous, and adductor muscles from the thigh and the gastrocnemius and tibialis anterior muscles were retrieved. The tissue samples were analyzed in a blinded fashion at Interactive Medical Technologies (Los Angeles, California). Regional perfusion was calculated as the ratio of microsphere content per gram of tissue between the ischemic and the nonischemic limb (14).
In vitro experiments. Human umbilical vein endothelial cells (HUVECs) (up to fifth passage) were grown in endothelial growth medium (EGM) (supplemented with 10% fetal bovine serum). Peripheral blood monocytes were isolated from human peripheral blood samples by centrifugation over Ficoll-Hypaque (Robbins Scientific Corp., Sunnyvale, California). The mononuclear cell layer was aspirated and washed twice in Hank’s balanced salt solution (HBSS). The CD14+ cells were obtained by positive selection with magnetic anti-CD14 microbeads according to the manufacturer’s instructions (Miltenyi Biotec, Auburn, California). Purity of the CD14+ cells was >95% as assessed by flow cytometry. Monocytes were cultivated no longer than 24 h in RPMI 1640 supplemented with 0.5% serum and 2 mmol/l glutamine. To avoid monocyte adhesion, hydrophobic culture dishes were used (In Vitro Systems, Osterode, Germany).

ADHESION ASSAY. Confluent HUVECs were stimulated with increasing concentrations of nicotine-free base (10^-4 to 10^-10 mol/l) or tumor necrosis factor (TNF)-alpha (10 ng/ml) (both from Sigma). Stimulated (4 h with nicotine or TNF-alpha) or unstimulated monocytes (10^6 cells in 100 μl) were incubated with HUVECs for 45 min on a rocker plate. Nonadherent cells were removed during three washing steps. Adhering cells were fixed in 2% paraformaldehyde and counted.

TRANSMIGRATION ASSAY. Confluent HUVECs on a porous polycarbonate membrane (pore diameter 3 μm; Millipore, Burlington, Massachusetts) coated with collagen I (10 μg/ml; Becton Dickinson, Franklin Lakes, New Jersey) were stimulated with TNF-alpha (10 ng/ml). Monocytes were labeled with CellTracker (Molecular Probes, Eugene, Oregon). The monocyte suspension (10^6 cells/ml) was added to the insert, whereas nicotine was placed in the lower chamber. After 4 h, the transmigrated monocytes were collected, fixed in 2% paraformaldehyde, and counted.

ANALYSIS OF CELL-ASSOCIATED FLUORESCENCE. The HUVEC and monocytes, respectively, were treated for 6 h with increasing concentrations of nicotine or TNF-alpha, washed with PBS, and trypsinized for 2 min. The cells were carefully scraped, separated by repeated pipetting, and washed with HBSS. The HUVECs were then incubated with fluorescein isothiocyanate-labeled mouse monoclonal anti-intercellular adhesion molecule-1 (ICAM-1) antibodies (Chemicon). Cells were fixed in 0.5% formaldehyde. Monocytes were simultaneously incubated with phycoerythrin-conjugated mouse anti-CD14 MAb (BD Pharmingen, San Diego, California) and fluorescein isothiocyanate-conjugated mouse monoclonal antibodies against CD11b (Mac-1) or CD11a (LFA-1; BD Pharmingen). Monocytes were fixed in 0.5% formaldehyde and washed with HBSS. The cell-associated fluorescence of 10,000 cells per sample was analyzed in a FACs Vantage flow cytometer (Becton Dickinson, San Jose, California). The MCP-1 was determined using a mouse JE/MCP-1 immunoassay (R&D Systems, Minneapolis, Minnesota).

Figure 1. Initial dose-escalating studies with nicotine concentrations ranging from 0.05 to 5.0 μg/kg/day with regional perfusion of the ischemic limb as determined by microsphere distribution as the primary end point. The nicotine concentrations of 5.0 μg/kg/day showed the strongest angiogenic response as compared with control.

Statistical analysis. All results for continuous variables are expressed as medians with 95% confidence intervals (CIs) (all figures show box plots with 75% and 95% CI). Comparisons between groups were analyzed by t test (two-sided) or analysis of variance for experiments with more than two subgroups. Post-hoc range tests and pairwise multiple comparisons were performed with the t test (two-sided) with Bonferroni adjustment. Comparison of categorical variables was generated by the Pearson chi-squared test. All analyses were performed with SPSS 10.0 (SPSS Inc., Chicago, Illinois). A value of p < 0.05 was considered statistically significant.

RESULTS

Rabbit model of hind-limb ischemia. Initial dose escalating studies with nicotine concentrations ranging from 0.05 to 5.0 μg/kg/day (n = 6 per group) revealed a maximum angiogenic response for the highest nicotine concentrations of 5.0 μg/kg/day as determined by microsphere distribution (Fig. 1). Accordingly, we used the latter concentration of nicotine in the final study investigating the effects of nicotine, bFGF, and vehicle on all selected end points.

Morphologic assessment. QUANTITATIVE ANGIOGRAPHY. Figure 2 shows representative internal iliac angiograms recorded at day 21 from PBS-, nicotine-, and bFGF-treated rabbits. The angiographic score for collateral vessel development in the medial thigh is summarized in Figure 3A. Before treatment (day 0), there were no significant differences among groups in angiographic score (p = 0.37). In both treatment groups, collateral artery development during 21-day follow-up was more marked compared with corresponding control groups. By day 21, the angiographic scores in both the nicotine-treated (74.0 [95% CI 59.5 to 88.4]) and bFGF-treated (73.6 [95% CI 69.0 to 78.2]) rabbits
exceeded that measured in the control group (52.1 [95% CI 46.4 to 57.8]; p = 0.001). There was no statistically significant difference in angiographic scores at 21 days between the nicotine-treated and bFGF-treated groups (p = 0.91).

HISTOLOGY. Tissue sections from the medial thigh muscles of the ischemic and nonischemic limbs were examined by light microscopy at day 21. Histologic evaluation after CD146 immunostaining revealed that the capillary density in the nicotine group (0.76 [95% CI 0.66 to 0.87]) and that in the bFGF-treated group (0.79 [95% CI 0.74 to 0.83]) were both significantly greater than values observed in the corresponding control group (0.35 [95% CI 0.31 to 0.39]; p < 0.001) (Fig. 3B). Analysis of the capillary density in the medial thigh muscle of the nonischemic limb showed no differences among groups (95% CI 0.22 [0.17 to 0.26] vs. 0.25 [95% CI 0.18 to 0.28]). Compared with the nonischemic hind limb, the number of collateral vessels in the ischemic limb of the control animals significantly increased from 12 (95% CI 9 to 21) to 37.5 (95% CI 31.8 to 43.2; p = 0.005) and the median size of those collateral from 118 μm (95% CI 106 to 133) to 187 μm (95% CI 166 to 209; p = 0.005) (Fig. 3C). Both nicotine and bFGF further increased the number of collateral vessels (50.3 [95% CI 43.8 to 56.9] and 46.2 [95% CI 37.6 to 54.7], respectively; p = 0.009) as well as the total vessel diameter (290 μm [95% CI 240 to 339] and 268 μm [95% CI 224 to 313], respectively; p = 0.001).

Figure 2. Selective internal iliac angiography at day 21-day in (A) control-, (B and C) nicotine-, and (D) basic fibroblast growth factor (bFGF)-treated rabbits. Nicotine was as potent as bFGF in stimulating arteriogenesis, although we observed more biologic variability in the response to nicotine. The two angiograms illustrate the range of response to nicotine.
Physiologic assessment. REGIONAL PERFUSION OF THE ISCHEMIC LIMB. Regional blood flow to ischemic limb muscles was analyzed in the hyperemic state with colored microspheres (Fig. 3D). Relative regional blood flow to the thigh (adductor) muscle was 49.6% (95% CI 44.6 to 54.6) versus 83.5% (95% CI 69.7 to 97.3) of the nonischemic limb (control vs. nicotine; $p < 0.001$). Relative regional blood flow to the distal lower limb (gastrocnemius) muscle was 35.9% (95% CI 28.8 to 43.0) versus 71.0% (95% CI 67.6 to 74.3) (control vs. nicotine; $p < 0.001$). In other words, microsphere studies indicated that the hyperemic flow values in the proximal and distal muscle groups were 1.7- and 2.0-fold, respectively, greater in nicotine-treated animals. No differences were observed in absolute regional flow among nonischemic muscles of both groups (flow to adductor muscle: 3.6 [95% CI 3.0 to 4.3] ml/min/100 g of tissue vs. 4.1 [95% CI 3.3 to 4.9] ml/min/100 g of tissue [$p = 0.58$]; flow to gastrocnemius muscle: 3.0 [95% CI 2.5 to 3.5]...
ml/min/100 g of tissue vs. 3.3 [95% CI 2.8 to 3.7] ml/min/100 g of tissue \( (p = 0.57) \). Results for bFGF-treated rabbits were similar to that observed for the nicotine-treated animals with 84.6% (95% CI 80.6 to 88.7) and 52.0% (95% CI 45.2 to 58.8) for the relative regional blood flow to the adductor and gastrocnemius muscles, respectively.

**INTRA-ARTERIAL DOPPLER GUIDE-WIRE MEASUREMENTS.** Doppler-derived blood flow was measured in the internal iliac artery of the ischemic and nonischemic limbs at day 0 and 21 (Fig. 3E). Resting blood flow and adenosine-stimulated blood flow in the ischemic limb were similar in all groups at day 0 (rest flow: nicotine 12.2 ml/min \[95% CI 10.2 to 14.8\]; bFGF 11.8 ml/min \[95% CI 9.9 to 13.8\]; control 12.5 ml/min \[95% CI 10.5 to 14.1\]; \( p = 0.72 \); hyperemic flow: nicotine 25.1 ml/min \[95% CI 19.7 to 28.2\]; bFGF 24.5 ml/min \[95% CI 20.1 to 28.5\]; control 25.7 ml/min \[95% CI 21.0 to 27.9\]; \( p = 0.87 \)). On day 21, nicotine-treated animals developed significantly enhanced flow in the ischemic limb after adenosine administration with 49.2 (95% CI 32.4 to 66.1) as compared with control (34.4 [95% CI 23.3 to 45.5]; \( p < 0.001 \)). Similar results were obtained for bFGF (45.0 [95% CI 33.1 to 56.8]). Relative to the contralateral, nonischemic limb, maximum flow in the internal iliac artery of the ischemic limb increased by 4.1-fold, by 3.3-fold, and by 2.0-fold in the nicotine, bFGF, and control groups, respectively (Fig. 3F; \( p < 0.001 \) vs. control for bFGF or nicotine). The resting blood flow was similar between nicotine- and bFGF-treated rabbits \( (p = 0.89) \), whereas the hyperemic flow tended to be higher in the nicotine group as compared with the bFGF group \( (p = 0.15) \). Iliac flow reserve, the ratio between blood flow at rest and after maximal pharmacologic stimulation, was 2.5 (95% CI 2.12 to 3.05) and 2.3 (95% CI 1.92 to 2.88) for the nicotine- and bFGF-treated rabbits, respectively. For nicotine-treated animals, this represented a statistically significant increase compared with control-treated animals (2.2 [95% CI 1.58 to 2.34]; \( p = 0.018 \)). Of note, ischemic complications (i.e., tissue loss) occurred in the vehicle \( (n = 3) \) but not nicotine group.

**In vitro studies. MONOCYTE ADHESION AND TRANSMIGRATION.** Adhesion and transmigration assays were performed with HUVECs and isolated human monocytes to determine the effects of nicotine on the interaction between monocytes and the endothelium. For unstimulated HUVECs the number of adhering monocytes was generally low (19 cells/high-power field [95% CI 15 to 32]; set as 100%). Binding of monocytes under other conditions is expressed as a percentage of this value. For TNF-alpha-stimulated HUVECs, adhesion of unstimulated monocytes was significantly higher (251% [95% CI 151 to 387]; \( p < 0.001 \)). In unstimulated HUVECs, previous exposure of monocytes to nicotine stimulated their adhesion in a dose-dependent manner with a maximum effect at \( 10^{-6} \) mol/l \( (231% [95% CI 206 to 274]; p < 0.001) \). Even stronger effects were observed when HUVECs were stimulated with nicotine or TNF-alpha, respectively, before nicotine-stimulated monocytes were added to the monolayer (572% and 621%, respectively; \( p < 0.001 \)). A transmigration assay served as an in vitro surrogate assay for monocyte diapedesis through the endothelium. As compared with control, nicotine stimulated monocyte transmigration in a concentration-dependent manner with a maximum effect observed for a nicotine concentration of \( 10^{-6} \) mol/l (398% [95% CI 312 to 481]; \( p < 0.001 \)). Mecamylamine, a specific and noncompetitive antagonist of the nAChR, and ICAM-1 blocking antibodies, respectively, abrogated those effects of nicotine on monocyte binding and subsequent transmigration (132% [95% CI 90 to 162]; \( p = 0.33 \)).

Cell-associated fluorescence as determined by flow cytometry cell demonstrated that stimulation of peripheral blood monocytes with nicotine resulted in a dose-dependent increase of CD11b (209% [95% CI 178 to 237]) and CD11a (165% [95% CI 151 to 194]) expression (Figs. 4A and 4B). Stimulation of HUVECs with nicotine caused a significant increase in ICAM-1 expression (308% [95% CI 264 to 328]). These results were confirmed by immunohistochemistry (data not shown). Mecamylamine abrogated the stimulatory effect of nicotine on adhesion molecule expression in both monocytes (Fig. 4B) and HUVECs (data not shown).

The MCP-1 measurements of the culture medium after 6 h of exposure to nicotine revealed a significant increase in MCP-1 concentration as compared with control (255% [95% CI 201 to 287]).

**DISCUSSION**

Nicotine enhances angiogenesis and arteriogenesis. The salient findings of this study are that: 1) acutely, nicotine increases angiogenesis and arteriogenesis in a model of hind-limb ischemia; 2) these effects are of the same magnitude as those obtained with bFGF; and 3) nicotine increases the expression of monocyte and endothelial adhesion molecules, increases the release of monocyte chemokine protein, and increases monocyte adherence and diapedesis, effects that may contribute to its enhancement of arteriogenesis.

Evidence of stimulated vessel formation in response to nicotine administration was observed at two levels. Histological studies documented an increase in vascularity at the capillary level, consistent with the classical definition of angiogenesis by Folkman (15). In addition, angiographic quantification showed that nicotine augmented the development of large collateral arteries. These angiographic and histologic effects were associated with increased perfusion as assessed by microsphere distribution and Doppler-derived blood flow in the internal iliac artery.

Direct intra-arterial infusion upstream from the occluded artery would be expected to optimize concentration and activity of nicotine in the local circulation of the affected limb. Indeed,
in our experiments, systemic cotinine levels, a major metabolite of nicotine and a reliable indicator of systemic nicotine exposure, were significantly lower than those levels usually observed in smokers (56 ng/ml; range 36 to 81 ng/ml) (16,17). At this relatively low dose of nicotine, its effects on angiogenesis and arteriogenesis are similar to the magnitude and time course as those achieved with the administration of vascular endothelial growth factor, bFGF, and MCP-1 (9,18–21).

Possible mechanisms. We have previously shown that the angiogenic effect of nicotine is mediated by endothelial nAChRs (5). However, there are other potential mechanisms by which nicotine might enhance revascularization in vivo. Nicotine is known to modulate the phenotype and enhance the proliferation of vascular smooth muscle cells (22,23), which could affect angiogenesis or remodeling of collateral channels. Nicotine could also directly stimulate circulating blood elements (e.g., monocytes) (24,25). Monocytes play an important role in collateral vessel formation by attaching to the activated endothelium and by invading the walls of pre-existing collateral vessels where they produce growth factors (21,26). The functional importance of monocyte adhesion during vascular remodeling was recently illustrated by the administration of ICAM blocking antibodies that markedly reduced collateral growth (27). Previous studies have demonstrated that this process can be promoted by several chemokines and growth factors (9,28,29). Here we show that nicotine can induce monocyte adhesion by more than two-fold if peripheral blood monocytes were pretreated with nicotine. The stimulation of peripheral blood monocytes was accompanied by a significant upregulation of the surface expression of both CD11b (Mac-1) and CD11a (LFA-1). If both the ECs and the peripheral blood monocytes were pretreated with nicotine, an almost six-fold increase in monocyte adhesion was observed. These results are comparable to those observed for TNF-alpha and previous findings for vascular endothelial growth factor, respectively (28). Adhesion of monocytes to the endothelium is followed by their migration into the vessel wall. A transmigration assay served as an in vitro surrogate assay for monocyte diapedesis through the vascular endothelium. Monocyte chemotaxis and transendothelial migration was significantly increased by nicotine. Additionally, we were able to demonstrate in vitro that nicotine induced the release of MCP-1 from ECs. Therefore, nicotine could promote monocyte extravasation both by directly stimulating monocytes (upregulation of integrins on monocytes) and indirectly by increasing MCP-1 release from the endothelium. It is possible that nicotine in part stimulates collateral growth via monocyte attraction and/or transmigration through the endothelium.

Nicotine, tobacco, and the double-edged sword of angiogenesis. The findings in this study seem counterintuitive. Tobacco use accelerates coronary and peripheral arterial disease. Tobacco cessation is a mainstay of therapy for these patients. Accordingly, it is surprising that nicotine would enhance angiogenesis in a model of ischemia. However, tobacco smoke is a complex mixture of over 4,000 chemical constituents, and the effect of nicotine delivered via the use of tobacco may be quite different. Indeed, there are several molecules in cigarette smoke that are toxic to ECs (e.g., cadmium, reactive oxygen species) (30). Indeed, Magers et al. (31) showed that tobacco smoke impaired angiogenesis in the reproductive organs of female hamsters. Similarly, extracts of tobacco smoke cause an abnormal patterning of blood vessels on chick chorioallantoic membranes (32).

However, it is possible that the angiogenic effect of nicotine contributes to the pathophysiologic effects of tobacco. As we have shown previously, the effect of nicotine to enhance angiogenesis accelerates neovascularization and plaque growth in a hypercholesterolemic mouse model, and

Figure 4. (A) Flow cytometry revealed that nicotine stimulated the expression of CD11b on peripheral blood monocytes. (B) The effect of nicotine to stimulate CD11b expression was dose-dependent and was abrogated by mecamylamine. Similar effects of nicotine on CD11a expression were observed by flow cytometry.
promoted tumor angiogenesis and tumor growth (5). These studies raise concerns about the chronic exposure to nicotine. Agonists of nAChRs are currently being studied for their beneficial effects in some neurologic disorders, including Alzheimer's disease, Parkinson's disease, and chronic pain (33–36). Angiogenesis is a double-edged sword (37,38), and the potent angiogenic effects of nicotine must now be taken into consideration by investigators who are studying the therapeutic potential of drugs that stimulate nAChRs.

Conclusions. We have demonstrated that acutely, nicotine stimulates angiogenesis and collateral growth and reduces the hemodynamic deficit in ischemic limbs. The angiogenic effect of nicotine may be mediated in part by its augmentation of monocyte integrin expression, MCP-1 release from ECs, and monocyte adhesion and transmigration through the endothelium. The effect of nicotine on angiogenesis and arteriogenesis is equivalent to that of bFGF. These effects of nicotine may play a role in tobacco-related diseases, such as tumors and atherosclerosis. Conversely, short-term and localized activation of this pathway may have application for disorders characterized by inadequate angiogenesis or arteriogenesis.

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