Effect of nicotine on vasoconstrictor and vasodilator responses in human skin vasculature

CLAIRE E. BLACK,1,2 NING HUANG,1 PETER C. NELIGAN,1,2 RONALD H. LEVINE,1,2 JOAN E. LIPA,1,2 STEVEN LINTLOP,3 CHRISTOPHER R. FORREST,1,2 AND CHO Y. PANG1,2,4

1Research Institute, The Hospital for Sick Children, and Departments of 2Surgery and 4Physiology, University of Toronto, and 3Center of Forensic Sciences, Toronto, Ontario M5G 1X8, Canada

Received 14 December 2000; accepted in final form 4 June 2001

Black, Claire E., Ning Huang, Peter C. Neligan, Ronald H. Levine, Joan E. Lipa, Steven Lintlop, Christopher R. Forrest, and Cho Y. Pang. Effect of nicotine on vasoconstrictor and vasodilator responses in human skin vasculature. Am J Physiol Regulatory Integrative Comp Physiol 281: R1097–R1104, 2001.—Our objective was to test the hypothesis that acute exposure of human skin vasculature to nicotine may have deleterious effects on endothelial function. Vasoconstriction and vasorelaxation in isolated perfused human skin flaps (∼8 × 18 cm) derived from dermolipectomy specimens were assessed by studying changes in skin perfusion pressure measured by a pressure transducer, and skin perfusion was assessed by a dermofluorometry technique (n = 4 or 5). It was observed that nicotine (10⁻⁷ M) amplified (P < 0.05) the norepinephrine (NE)-induced concentration-dependent (10⁻⁷–10⁻⁵ M) increase in skin vasoconstriction compared with the control. This amplification effect of nicotine in NE-induced skin vasoconstriction was not blocked by the nicotine-receptor antagonist hexamethonium (10⁻⁶ M) or the cyclooxygenase inhibitor indomethacin (10⁻⁵ M). It was also observed that ACh and nitroglycerin (NTG) elicited a concentration-dependent (10⁻⁸–10⁻⁵ M) vasorelaxation in skin flaps preconstricted with 8 × 10⁻⁷ M of NE. The vasorelaxation induced by ACh was attenuated (P < 0.05) in the presence of nicotine (10⁻⁷ M) compared with the control. However, skin vasorelaxation induced by NTG was not affected by nicotine (10⁻⁷ M). ACh and NTG are known to induce endothelium-dependent and -independent vasorelaxation, respectively. The present findings were interpreted to indicate that acute exposure of human skin vasculature to nicotine was associated with 1) amplification of NE-induced skin vasoconstriction and 2) impairment of endothelium-dependent skin vasorelaxation. Cyclooxygenase products and nicotine receptors blocked by hexamethonium were not involved in the amplification of NE-induced skin vasoconstriction by nicotine. These findings may provide further insight into the pathogenesis of skin vasospasm in skin flap surgery and skin ischemic disease associated with cigarette smoking or use of smokeless tobacco.

norepinephrine; acetylcholine; nitroglycerin

THE ASSOCIATION OF CIGARETTE smoking or use of smokeless tobacco (e.g., chewing tobacco) with arteriosclerotic peripheral vascular disease and thromboangiitis obliterans (Buerger’s disease) has been identified for some time (16, 21, 31, 38, 43). More recently, there is also the clinical impression that cigarette smoking increases the incidence of skin ischemic necrosis in skin flap surgery (45). This clinical impression is supported by the observations that cigarette smoke intensified skin ischemic necrosis in skin flap surgery in the rat and hamster (11, 23, 28, 42). Skin vasospasm associated with cigarette smoking and or use of smokeless tobacco implies that the by-products of tobacco may have detrimental effects on the skin vasculature, resulting in reduction in skin circulation, but the mechanism is unclear. Epidemiological studies on the deleterious effects of cigarette smoking in humans indicated that the risk in developing cigarette smoking-related cardiovascular disease was related to the nicotine content of the cigarette (2, 3, 18). Therefore, much of the research on the pathogenesis of cigarette smoking-related cardiovascular diseases has been focused on the deleterious effect of nicotine on the vasculature. To date, there is experimental evidence to indicate that chronic nicotine treatment has deleterious effects on the hemodynamics of the cardiovascular system either by causing direct injury to the blood vessel wall (8, 19, 54) or by modulating the synthesis and/or release of vasactive neurohumoral substances (1, 49, 50) to promote vasospasm and platelet aggregation.

Of particular interest to us is the effect of nicotine on skin circulation. With the use of skin flap models, we have demonstrated that chronic nicotine treatment in rats and pigs (13–15) mimicked the deleterious effect of cigarette smoke in increasing the extent of skin ischemic necrosis in skin flap surgery in the rat and hamster (11, 23, 28, 42). This deleterious effect was associated with an increase in skin flap content of norepinephrine (NE) and a decrease in skin flap blood flow (13–15). More recently, it has been reported by other investigators that acute nicotine treatment selectively potentiated NE-induced arteriole contraction in the hamster cheek pouch. This potentiation effect was attributed to the nicotine-induced impairment of endothelial function in the arterioles (33). Most recently, it

http://www.ajpregu.org 0363-6119/01 $5.00 Copyright © 2001 the American Physiological Society R1097

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
has been demonstrated that acute nicotine treatment mimicked the cigarette smoking-induced endothelial dysfunction in the human dorsal hand vein. Specifically, local or transdermal administration of nicotine at a dose reproducing plasma concentration observed during cigarette smoking impaired endothelium-dependent vasodilation in human dorsal hand veins (9, 47). All these recent findings may have important implications for the direct vascular effect of nicotine, which contributes to the pathogenesis of vasospasm in skin ischemic disease and in skin flap surgery. To our knowledge, no studies have been conducted to examine the effect of nicotine on skin vascular function, especially in human skin vasculature. Therefore, the objective of this project was to test the hypothesis that nicotine may have deleterious effects on the endothelial function in human skin vasculature. To this end, we used the established isolated perfused human skin flap model (25, 26, 32) to investigate the local effect of nicotine on vasoconstrictor and vasodilator responses in human skin vasculature.

MATERIALS AND METHODS

Source of Human Skin Specimens

The abdominal pannus excised from patients undergoing dermolipectomy serves no purpose to the patient and is normally disposed of by incineration. A clinical protocol was approved for the design of skin flaps (−8 × 18 cm) from the excised pannus for in vitro skin perfusion experiments. At the end of each experiment, the skin specimen was returned to the Department of Pathology for incineration in the normal manner.

The median age of the patients was 44 yr (range 26–65 yr), and all these patients were female. The skin specimens accepted for this project did not have scars, lesions, or infection, and the patients were not known to smoke cigarettes or to have any systemic disease.

Skin Flap Model and Cannulation Technique for In Vitro Skin Perfusion

The anatomy and design of the human skin flap model used in the present studies were described by us previously (32). This skin flap model resembled clinical skin free flaps used for reconstructive surgery. This skin flap model has been validated and studied with various kinds of vasodilator and vasoconstrictor drugs (25, 26, 32).

The width and length of this skin flap model were −8 × 18 cm, respectively. The vascular pedicle in the proximal end of the skin flap consisted of a paired perforator artery and vein (0.5- to 1.5-mm diameter), which were cannulated with 22- or 24-gauge angiocatheter, depending on vessel size. Perfusion buffer containing 10 U/ml of heparin was gently instilled through the arterial angiocather with a 3-ml syringe until venous outflow was observed, thus confirming that the vasculature of the skin flap was satisfactory for an in vitro perfusion experiment. All perfusion experiments in the present studies were initiated within 2 h of excision of skin specimens. It was previously demonstrated that the skin flap was thereafter metabolically and physiologically stable for at least 5 h of in vitro perfusion (26).

Skin Perfusion Technique

The skin flap with its cannulated arterial and venous perforator was placed on an aluminum mesh and was subsequently connected to the commercially available MX Amber perfusor apparatus (model Two-Ten; MX International, Aurora, CO). The perfusate consisted of modified Krebs-Henseleit buffer with the following composition (in mM): 100 NaCl, 4.6 KCl, 1.10 NaH2PO4, 1.20 MgSO4·7H2O, 2.25 CaCl2, 30 NaHCO3, and 13 glucose. BSA (Cohen fraction V) was added to the buffer for a final concentration of 6.5%. The buffer was then stirred and filtered (Whatman no. 40) and equilibrated within the reservoir chamber of the perfusion apparatus with 95% O2 and 5% CO2 at 37 ± 0.1°C, pH 7.40 ± 0.03 mmHg, and Po2 450 ± 16 mmHg. The Po2 of the perfusate collected at the venous outflow was 180 ± 25 mmHg. This observation indicated adequate supply of oxygen to the skin flap as there was minimal arteriovenous shunt flow (<1%) in this skin flap model (27). An adjustable-rate pump (model 7014; Cole-Parmer Instrument, Vernon Hills, IL) was used to deliver perfusate, which passed through a bubble trap in the reservoir to the arterial catheter of the skin flap. A three-way connector linked the tubing from the pump to the flap and allowed for parallel connection of a pressure transducer (AB high-performance pressure transducer; Data Instruments, Lexington, MA). The transducer output continuously displayed the perfusion pressure on a digital monitor (Trandicator II 621A digital strain gauge; Doric Scientific, San Diego, CA) and a chart recorder (Linearcorder WR 3101; Graphtec).

The buffer flow rate was adjusted (2.0 ± 0.4 ml/min) to achieve a stable baseline perfusion pressure of 45–50 mmHg. A baseline of −50 mmHg was chosen because results from previous studies with this human skin flap model revealed it to provide good tissue perfusion with minimal leakage and edema formation of less than 10% water retention (25, 32). In addition, it has been estimated that a perfusion pressure of 50 mmHg, when Krebs buffer with an albumin concentration of 65 g/l is used, is equivalent to the perfusion pressure of −90 mmHg in a whole blood perfusion (4). A perfusion pressure of 50 mmHg with a similar perfusate was also successfully used in studies of the more aerobically active isolated perfused cat hindlimb (53).

A 45-min stabilization period was allowed at the beginning of each experiment. The surface temperature (−34°C) of the skin flap was monitored using a thermistor probe (YS1 series 400; Yellow Springs Instruments, Yellow Springs, OH) connected to a microcomputer thermometer (Series 084202; Cole-Parmer Instrument). Drugs were infused intra-arterially through a sidearm port driven by a separate pump at adjustable rates (model P-1; Pharmacia LKB). Under the constant flow condition in which the pump rate remained constant, a change in perfusion pressure in response to drug administration is indicative of a change in vascular resistance of the skin flap. The change in perfusion pressure induced by drug treatment in each skin flap was standardized by its baseline perfusion pressure and is expressed as a percentage of baseline perfusion pressure.

Criteria for Rejection of Skin Flap

A skin flap was deemed to be vascularly reactive if it demonstrated a concentration-dependent increase in perfusion pressure to NE. At the end of the last dose of NE in each experiment, ACh (10−5 M) was used to test endothelium-dependent relaxation. Finally, 0.2 ml of fluorescein dye (20 mg) was infused into each flap to characterize the area of skin
perfusion. Any skin flap that did not respond to the concentration-dependent vasoconstrictor effect of NE and vasodilator effect of ACh and showed <6-cm length in dye stain (perfusion) was not included in this study.

Surface Dermofluorometry Technique for Assessment of Skin Perfusion in Isolated Perfused Skin Flaps

The dermofluorometry technique for indirect assessment of in vivo dermal perfusion has been validated against the radioactive microsphere technique in the pig (51). Dermofluorometry has also been applied to the present isolated perfused human skin flap model in vitro (24, 25, 32). In the present study, dermofluorometry was used to corroborate the observation of skin vasoconstriction assessed by measurement of perfusion pressure. Specifically, confluent circles of 1-cm diameter were marked along the longitudinal midline of the skin flap surface. After a 45-min stabilization period, the background fluorescence in each circular skin area was measured (fluorescence unit) using a dermofluorometer (Fluorescan Unit; Santa Barbara Technology, Santa Barbara, CA). Fluorescein dye with a final concentration of $3 \times 10^{-5}$ M was then infused into the skin flap for 4 min, and fluorescence in each circular area was measured again. A washout period of 15 min was allowed, and the background fluorescence was taken again. After the perfusion pressure had stabilized subsequent to drug infusion for study of skin vascular reactivity, fluorescein dye infusion was repeated, and skin fluorescence was measured again. The difference in fluorescence units for each circular skin area between the background and postfluorescein dye infusion defined as the net fluorescence units for that area. The total dye fluorescence is the sum of all net fluorescence units measured from all circular skin areas along the midline of the skin flap.

Biochemicals

All reagents and drugs were purchased from Sigma Chemical (Oakville, Ontario) except the following: NE from SABEX Pharmaceutical (Boucherville, Quebec) and fluorescein dye (fluorescein sodium 10%) from Dioptic Laboratories (Markham, Ontario). Indomethacin, a cyclooxygenase inhibitor, was dissolved in 200 µl ethanol before mixing with buffer solution. This concentration of alcohol did not affect the baseline perfusion pressure. Other drugs used in the present studies were dissolved in buffer. Purified water (Milli-Q Water System, Bedford, MA) was used for making perfusion buffer. Fresh perfusion buffer and drug solutions were made on the day of experiment. The drugs were stored at 4°C before use.

Experimental Protocols

 Protocol 1: to investigate the effect of nicotine on NE-induced skin vasoconstriction by assessment of perfusion pressure in isolated perfused human skin. Cumulative concentration-dependent ($10^{-7}$–$10^{-5}$ M) vasoconstrictor effects of NE on skin perfusion pressure were studied in the absence and presence of $10^{-7}$ M nicotine in isolated human skin flaps perfused with a constant flow rate of Krebs buffer. NE was used in this study because it was reported that nicotine selectively potentiated the vasoconstrictor effect of NE in hamster cheek pouch arterioles (33).

 Protocol 2: to investigate the effect of nicotine on NE-induced skin vasoconstriction by assessment of skin perfusion in isolated perfused human skin. Skin perfusion was assessed by the dermofluorometry technique to corroborate the observation of skin vasoconstriction assessed by measurement of perfusion pressure in the preceding study. Specifically, the NE-induced ($8 \times 10^{-7}$ M) decrease in dermal perfusion in isolated perfused human skin flaps was assessed in the absence and presence of $10^{-7}$ M of nicotine, using the dermofluorometry technique. Total dye fluorescence was assessed at the end of: the stabilization period (baseline); 15-min infusion of vehicle; and 15-min infusion of NE in the absence and presence of $10^{-7}$ M of nicotine.

 Protocol 3: to investigate the role of nicotine receptors and endogenous cyclooxygenase products in the effect of nicotine on NE-induced skin vasoconstriction in isolated perfused human skin. The effect of nicotine ($10^{-7}$ M) on the cumulative concentration-dependent vasoconstrictor effect of NE on skin perfusion pressure was studied in the absence and presence of the nicotine-receptor antagonist hexamethonium ($10^{-6}$ M). Hexamethonium infusion was started 45 min before nicotine infusion and followed by NE infusion 45 min later.

 In a separate study, all skin flaps were pretreated with $10^{-5}$ M of indomethacin. The vasoconstrictor effect of NE ($8 \times 10^{-7}$ M) on skin perfusion pressure was studied in the absence and presence of $10^{-7}$ M of nicotine. Infusion of indomethacin and nicotine was started 45 min before NE infusion.

 Protocol 4: to investigate the effect of nicotine on ACh- or nitroglycerin-induced vasorelaxation in isolated perfused human skin. The effect of nicotine ($10^{-7}$ M) on cumulative concentration-dependent vasorelaxation induced by ACh or nitroglycerin (NTG) was studied in isolated perfused human skin flaps preconstricted with $8 \times 10^{-7}$ M of NE. ACh and NTG were used in this study because it is well known that ACh elicits endothelium-dependent vasorelaxation and NTG elicits endothelium-independent vasorelaxation (35–37).

Statistics

All values are expressed as means ± SE. The number of observations and the specific statistical test used in each study are indicated in the legend of each figure. The level of significance for all tests was set at $P < 0.05$.

RESULTS

Effect of Nicotine on NE-Induced Skin Vasoconstriction Assessed by Perfusion Pressure in Isolated Perfused Human Skin Flaps

Intra-arterial infusion of nicotine ($10^{-7}$ M) was started 45 min before infusion of NE in the isolated perfused human skin flap. Infusion of nicotine alone did not have any effect on the baseline perfusion pressure. The baseline perfusion pressures before (47.6 ± 1.7 mmHg) and after (48.3 ± 1.0 mmHg) nicotine infusion were not significantly different. NE elicited an increase in perfusion pressure in isolated perfused human skin flaps in a concentration-dependent ($10^{-8}$–$10^{-5}$ M) manner (Fig. 1). This increase in skin perfusion pressure induced by NE was significantly higher ($P < 0.05$) in the presence of $10^{-7}$ M of nicotine compared with the control, thus indicating that nicotine amplified the skin vasoconstrictor effect of NE.
Effect of Nicotine on NE-Induced Skin Vasconstriction Assessed by Dermofluorometry Technique in Isolated Perfused Human Skin Flaps

The amplification effect of nicotine on NE-induced vasconstriction was further assessed by using the dermofluorometry technique. The total dye fluorescence in the vehicle-treated skin flaps was similar to the baseline perfusion (100 ± 4%). Infusion of $8 \times 10^{-7}$ M of NE significantly ($P < 0.05$) reduced the total dye fluorescence to 37 ± 4% ($P < 0.05$) of the control (vehicle). In the presence of $10^{-7}$ M of nicotine, this same concentration of NE further reduced the total fluorescence to 20 ± 5% ($P < 0.05$) of the control (Fig. 2). Thus the present results from the dermofluorometry study supported the observation from the preceding study that nicotine amplified the skin vasoconstrictor effect of NE by assessment of perfusion pressure.

Role of Nicotine Receptors and Endogenous Cyclooxygenase Products in Effect of Nicotine on NE-Induced Vasconstriction in Isolated Perfused Human Skin Flaps

In the preceding study, it was observed that the NE-induced increase in skin perfusion pressure in human skin was significantly ($P < 0.05$) higher in the presence of $10^{-7}$ M of nicotine compared with the control (Fig. 1). Here, it was observed that the increase in skin perfusion pressure induced by combined NE and nicotine was similar in skin flaps with or without pretreatment with the nicotine-receptor antagonist hexamethonium ($10^{-6}$ M) (Fig. 3). Therefore, nicotine receptors blocked by hexamethonium were not involved in the amplification effect of nicotine in NE-induced increase in perfusion pressure.

In a separate study, all skin flaps were pretreated with indomethacin ($10^{-5}$ M). It was observed that the NE-induced increase in perfusion pressure was still significantly ($P < 0.05$) higher than that of NE alone (Fig. 4). This observation is interpreted to indicate that the endogenous cyclooxygenase products were not involved in the

Fig. 1. Effect of nicotine ($10^{-7}$ M) on norepinephrine (NE)-induced skin vasconstriction. Values are means ± SE, $n = 4$. The cumulative concentration-dependent increase in perfusion pressure elicited by NE in isolated perfused human skin flaps was significantly higher in the presence of nicotine compared with the control; analysis of variance with repeated measures, $P < 0.05$.

Fig. 2. Effect of nicotine ($10^{-7}$ M) on NE-induced ($8 \times 10^{-7}$ M) decrease in dermal perfusion. Dermal perfusion was assessed by measuring the total dye fluorescence in the skin flap using a dermofluorometry technique. Total dye fluorescence is expressed as a percentage of the control baseline before drug treatment. Values are means ± SE, $n = 4$. Means without a common letter are significantly different (a > b > c); 1-way analysis of variance followed by Duncan’s multiple range test for comparison of means, $P < 0.05$.

Fig. 3. Role of nicotine receptors in the effect of nicotine ($10^{-7}$ M) on NE-induced skin vasconstriction. Values are means ± SE, $n = 4$. The cumulative concentration-dependent increase in perfusion elicited by NE and nicotine was not significantly different in the presence or absence of the nicotine-receptor antagonist hexamethonium ($10^{-6}$ M); analysis of variance with repeated measures.

Fig. 4. Role of cyclooxygenase products in the effect of nicotine ($10^{-7}$ M) on NE-induced skin vasconstriction. Values are means ± SE, $n = 4$. All isolated perfused skin flaps were pretreated with the cyclooxygenase inhibitor indomethacin ($10^{-5}$ M). The increase in perfusion pressure induced by NE and nicotine was significantly higher than NE alone; Mann-Whitney $U$ test, $P < 0.05$. 
amplification effect of nicotine on NE-induced increase in perfusion pressure.

**Effect of Nicotine on ACh- and NTG-Induced Vasorelaxation in Isolated Perfused Human Skin Flaps Preconstricted with NE**

ACh elicited a concentration-dependent relaxation in isolated perfused human skin flaps preconstricted with $8 \times 10^{-7}$ M of NE (Fig. 5). The concentration-dependent vasorelaxation induced by ACh in isolated perfused human skin flaps preconstricted with NE was significantly $(P < 0.05)$ reduced in the presence of $10^{-7}$ M of nicotine compared with the control (Fig. 5).

NTG also elicited a concentration-dependent vasorelaxation in isolated perfused human skin flaps preconstricted with $8 \times 10^{-7}$ M of NE. However, the concentration-dependent vasorelaxation induced by NTG in human skin flaps preconstricted with $8 \times 10^{-7}$ M of NE was not significantly different in the absence or presence of $10^{-7}$ M of nicotine (Fig. 6).

**DISCUSSION**

**Major Findings in the Present Studies**

We used the isolated perfused human skin flap model to investigate the acute local effect of nicotine on vasoconstrictor and vasodilator responses in human skin vasculature. We observed that 1) nicotine amplified the concentration-dependent skin vasoconstrictor effect of NE; 2) nicotine attenuated endothelium-dependent skin vasorelaxation induced by ACh in NE-preconstricted skin vasculature; 3) nicotine did not affect the endothelium-independent vasorelaxation induced by NTG in NE-preconstricted skin vasculature; and 4) cyclooxygenase products and nicotine receptors blocked by hexamethonium were not involved in the amplification of NE-induced skin vasoconstriction by nicotine. These observations led us to speculate that exposure of human skin vasculature to nicotine may impair the endothelial vasorelaxation function in the vasculature.

**Levels of Nicotine and NE Used for the Study of Endothelial Function**

Studies on nicotine pharmacokinetics in cigarette smokers have shown that peak levels of plasma nicotine range from $\sim 30$ to $50$ ng/ml (7, 12). The half-life of plasma nicotine due to metabolism is $\sim 2.2$ h (12). It has also been reported that most cigarette smokers tend to modify their smoking behavior (e.g., depth of puffing or inhalation, and frequency of smoking) to maintain fairly constant plasma levels of nicotine (12). The average peak plasma levels of nicotine in smokeless tobacco users have also been reported to range from $\sim 22$ to $30$ ng/ml (17). The level of nicotine in the perfusion buffer used in the present studies was $10^{-7}$ M (16.2 ng/ml), which is well within the range of plasma levels of nicotine in cigarette smokers and users of smokeless tobacco.

We previously observed that NE elicited concentration-dependent skin vasoconstriction over a range of $10^{-7}$ to $10^{-5}$ M in isolated perfused pig skin flaps (44). The mean concentration of NE required to produce half-maximal increase in perfusion pressure ($EC_{50}$) was $1.1 \times 10^{-7}$ M. Similar results were reported by other investigators for isolated perfused pig skin flaps (46). In addition, the skin contents of NE in rat and pig skin flaps were also in the range of $10^{-7}$ to $10^{-6}$ M (15, 22). Therefore, $10^{-7}$- to $10^{-5}$-M concentrations of NE were used for the present studies.

**Experimental Model for the Study of Nicotine on Vasoconstrictor and Vasodilator Responses in Human Skin Vasculature**

Many of the in vivo cardiovascular effects observed after cigarette smoking, such as increases in blood pressure and heart rate, result from activation of the sympathoadrenal system by nicotine (5, 6). Thus the
net effect of nicotine on vascular tone is determined by the balance between peripheral and central sites of action of nicotine, and the local vascular effect of nicotine cannot be distinguished. Recently, the human dorsal hand vein model was used to study the acute local effect of nicotine on vascular endothelial function with minimal systemic effect. Specifically, physiological saline with or without nicotine and/or vasoactive drugs was infused continuously into a dorsal hand vein to study the local vasoconstriction and vasorelaxation effects of these drugs by measuring the changes in diameters of the dorsal hand vein (9). So far, there is no study on the local effect of nicotine on human skin vasculature relevant to vasospasm in skin ischemic disease and in skin flap surgery. Our isolated perfused human skin flap model permitted local intra-arterial infusion of nicotine and drugs into the skin vasculature to study local vasoconstrictor and vasodilator responses (26, 32). Krebs buffer instead of whole blood was used for perfusate in the present studies so that the local effect of nicotine on endothelial function could be investigated in the absence of blood cells and circulating neurohumoral vasoactive substances.

**Local Effect of Nicotine on Vasoconstrictor and Vasodilator Responses in Human Skin Vasculature**

In the present studies, we have obtained evidence to indicate that nicotine amplified skin vasoconstriction induced by NE. Specifically, NE-induced skin vasoconstriction assessed by perfusion pressure or dermal perfusion was significantly increased in the presence of $10^{-7}$ M of nicotine compared with NE alone (Figs. 1 and 2).

There is also evidence from the present studies to indicate that nicotine amplified the NE-induced skin vasoconstriction independent of nicotine receptors blocked by hexamethonium and endogenous cyclooxygenase products. This interpretation is based on the following observations. The nicotine-receptor antagonist hexamethonium did not attenuate the concentration-dependent increase in perfusion pressure induced by combined NE and nicotine in isolated perfused human skin flaps (Fig. 3). Moreover, the cyclooxygenase inhibitor indomethacin did not block the amplification effect of nicotine in NE-induced increase in perfusion pressure in isolated perfused human skin flaps (Fig. 4).

Furthermore, we have also obtained evidence to indicate that nicotine attenuated endothelium-dependent but not endothelium-independent vasorelaxation in human skin. Specifically, the concentration-dependent vasorelaxation effect of ACh in isolated perfused human skin flaps preconstricted with NE was significantly reduced in the presence of $10^{-7}$ M of nicotine compared with ACh alone (Fig. 5). However, the same nicotine treatment did not affect the concentration-dependent vasorelaxation induced by NTG in isolated perfused human skin flaps preconstricted with NE (Fig. 6).

**Mechanism of Action of Nicotine in Vasoconstrictor and Vasodilator Responses in Human Skin Vasculature**

We previously observed in isolated perfused pig skin flaps that NE-induced concentration-dependent vasoconstriction was enhanced in the presence of the nitric oxide synthase (NOS) inhibitor L-NNA ($L$-nitro-$L$-arginine ($L$-NNA), and this skin vasoconstriction was further enhanced in the presence of both $L$-NNA and the cyclooxygenase inhibitor indomethacin (15). We also previously observed that $L$-NNA attenuated the vasorelaxation effect of ACh in isolated perfused human skin flaps preconstricted with NE (25). These observations were interpreted to indicate that the endothelium of skin vasculature could be stimulated to synthesize and/or release vasorelaxing factors such as NO and prostacyclin (PGI2) to counteract the vasoconstrictor effect of NE or to mediate the vasorelaxation effect of ACh. Experimental evidence from other laboratories is also available to indicate that contractions of isolated blood vessels caused by NE or stimulation of adrenergic nerves were associated with simultaneous release of endothelium-derived NO and PGI2 (10, 48). The mechanisms were not investigated in these studies. However, it is known that NE activates $\alpha_1$-adrenoceptors in smooth muscle cells to cause vasoconstriction. NE also activates $\alpha_2$-adrenoceptors in the endothelium to stimulate synthesis/release of NO and PGI2 (52). ACh is also known to activate muscarinic receptors in the endothelium to stimulate synthesis/release of NO (52). These previous observations provide insight into the mechanism of nicotine in amplification of NE-induced skin vasoconstriction (Fig. 1) and in attenuation of ACh-induced vasorelaxation (Fig. 5) observed in isolated perfused human skin flaps in the present studies. Specifically, we speculate that nicotine might have inhibited the NOS activity in the endothelium to cause a reduction in synthesis/release of NO in the endothelium. Therefore, there was less NO to counteract NE-induced skin vasoconstriction or to mediate ACh-induced vasorelaxation. We further speculate that the mechanism of action of nicotine did not involve hexamethonium-sensitive receptors or cyclooxygenase products, because the amplification effect of nicotine on NE-induced skin vasoconstriction was not attenuated by hexamethonium or indomethacin (Figs. 3 and 4).

This line of reasoning may also explain why nicotine did not affect the baseline perfusion pressure of isolated perfused human skin flaps. Specifically, the decrease in NO synthesis due to inhibition of NOS activity in the endothelium by nicotine could have been compensated by an increase in PGI2 synthesis at baseline level; thus the baseline perfusion pressure remained unchanged. However, this small increase in PGI2 synthesis could not compensate for the decrease in NO synthesis in the endothelium caused by nicotine in NE-induced skin vasoconstriction or in ACh-induced vasorelaxation. There is in vivo evidence to support this speculation as well. Specifically, it has been reported that a similar level of nicotine ($14.0 \pm 1.6$ ng/ml)
did not affect the baseline diameter of the hamster cheek pouch arterioles, but this same level of nicotine potentiated the NE-induced vasoconstriction in the hamster cheek pouch in vivo (33, 34).

Potential Limitations

It is important to point out that we only studied the acute effect of nicotine on vasoconstrictor and vasodilator responses in NE-preconstricted human skin vasculature. It is not known if these responses will also be seen in chronic nicotine treatment and/or with vasoconstrictors other than NE. There is evidence to indicate that nicotine impaired ACh-induced endothelium-dependent vasorelaxation in both acute and chronic nicotine treatment in hamster cheek pouch arterioles (35–37); thus there is evidence for us to speculate that the acute and chronic deleterious effect of nicotine on endothelial function is similar. However, the amplification effect of nicotine on vasoconstriction in hamster cheek pouch arterioles seemed to be selective to NE (33).

So far, we have demonstrated that nicotine amplified the vasoconstrictor effect of NE and impaired the ACh-induced endothelium-dependent vasorelaxation in human skin vasculature in vitro. These findings are in line with the in vivo observations reported by other investigators that local administration of nicotine potentiated the vasoconstrictor effect of NE (33) and impaired the endothelium-dependent vasorelaxation in hamster cheek pouch arterioles (35–37) and in human dorsal hand veins (9, 47). However, it is unclear why these vascular effects of nicotine were not observed in tail artery ring segments and isolated perfused mesenteric vasculature obtained from rats with chronic nicotine treatment or in coronary artery segments obtained from dogs with chronic nicotine treatment (29, 39). The differences in these results could reflect differences in vasculature, experimental design, and levels of nicotine used.

Perspectives

The effect of nicotine on amplification of NE-induced vasoconstriction and impairment of endothelium-dependent vasorelaxation observed in human skin in the present studies may have important clinical implications in the pathogenesis of skin vasospasm in skin flap surgery and skin ischemic disease. For example, there is the clinical impression that cigarette smoking increases the incidence of skin ischemic necrosis in skin flap surgery (45). We have previously demonstrated that chronic nicotine treatment in rats and pigs (13–15) mimicked the detrimental effect of cigarette smoking in increasing the extent of skin flap ischemic necrosis, and this detrimental effect was associated with an increase in skin content of NE, presumably released by nerve endings in the skin (15). Here, we demonstrated for the first time in human skin that nicotine may also amplify the skin vasoconstrictor effect of NE, probably by inhibition of NOS activity in the endothelium to reduce NO synthesis. Together, these observations provide insight into the pathogenesis of intensified skin vasospasm induced by cigarette smoking in skin flap surgery. However, we have also previously observed in rats that the detrimental effect of chronic nicotine treatment on skin flap blood flow and viability was reversible and was not encountered when nicotine was withheld 2 wk before skin flap surgery (14). This information may also be relevant to certain states of ischemic skin disease as it has been reported that resolution of symptoms such as skin vasospasm and pain in Buerger’s disease (thromboangiitis obliterans) induced by use of tobacco could be achieved in some patients by abstinence from tobacco use and a regimen of a vasodilating drug (20, 30, 40, 43).

In summary, we demonstrated for the first time that acute nicotine treatment amplified the skin vasoconstrictor effect of NE and impaired the endothelium-dependent vasorelaxation in isolated perfused human skin flaps preconstricted with NE. Cyclooxygenase products and nicotine receptors blocked by hexamethonium were not involved in the amplification of NE-induced skin vasoconstriction by nicotine. These observations lent support to our hypothesis that nicotine may have deleterious effects on endothelial function in skin vasculature, but the mechanism has yet to be investigated.

The authors thank D. Whitwell and D. McIntyre for typing this manuscript.

This research project was supported by an operating grant from The Smokeless Tobacco Research Council (Grant 0784) and the Canadian Institute of Health Research (Grant MOP-8048) to C. Y. Pang.

REFERENCES


AJP-Regulatory Integrative Comp Physiol • VOL 281 • OCTOBER 2001 • www.ajpregu.org
18. Mayhan WG. NICOTINE AND ENDOTHELIAL FUNCTION
23. Mayhan WG. NICOTINE AND ENDOTHELIAL FUNCTION