Nicotine increases initial blood flow responses to local heating of human non-glabrous skin

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Nicotine affects the regulation of skin blood flow (SkBF), but the mechanisms involved are not well understood. We tested the hypothesis that acute exposure to nicotine inhibits both the initial neurally mediated component and the later sustained component of SkBF responses to local heating of non-glabrous skin in humans. SkBF (measured by laser-Doppler) responses to local heating of forearm skin from 32 to 42°C were measured in 11 chronic smokers. Heating occurred at one site over 15 min (RAMP) and over 90 s (STEP) at another site, and was maintained for an additional 30 min. STEP heating was also applied to a site pretreated with bretylium via iontophoresis to inhibit noradrenergic neurotransmission. Responses were measured before and after acute administration of nicotine via cigarettes or nasal spray in two experimental sessions. Nicotine decreased resting skin blood flow ($P < 0.05$); this response was inhibited by bretylium. During RAMP, nicotine increased the initial SkBF at 42°C (by ∼12%, $P < 0.05$). For STEP, nicotine increased the initial peak response (by ∼25%, $P < 0.05$), and decreased the sustained plateau value (by ∼10%, $P < 0.05$). In skin pretreated with bretylium, the increase caused by nicotine in the initial peak value persisted, but the plateau value was not different from pre-nicotine. These data suggest that in abstinent cigarette smokers, nicotine augments initial responses to both gradual and rapid non-painful heating of non-glabrous skin by sensitizing the sensory nerves that mediate the axon reflex associated with rapid vasodilatation. In contrast, nicotine decreases SkBF responses to prolonged heating by activating noradrenergic nerves.

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Local heating of human non-glabrous skin increases skin blood flow (SkBF) via contributions from both local axon reflexes and the local release of mediators (Magerl & Treede, 1996; Kellogg *et al.* 1999; Minson *et al.* 2001; Stephens *et al.* 2001). Recent evidence suggests that it is possible to distinguish between these components of human SkBF responses using rapid, non-painful increases in local skin temperature (Kellogg *et al.* 1999; Minson *et al.* 2001). The neural component of this response, measured as an initial peak dilator response, is caused by the release of vasoactive peptides such as calcitonin gene-related peptide (CGRP) from a subpopulation of C-fibre nociceptive afferents via an axon reflex (Magerl & Treede, 1996; Schmelz *et al.* 1997; Klede *et al.* 2003). The neural element transducing this temperature-regulated mediator release is likely in the transient receptor potential (TRPV) channel subfamily (Caterina *et al.* 1997; Zygmunt *et al.* 1999; Schwarz *et al.* 2000; Xu *et al.* 2002), which includes the vanilloid receptor stimulated by capsaicin. The later component of sustained vasodilatation is caused, at least in part, by the local release of nitric oxide (NO) by mechanisms that are unknown (Kellogg *et al.* 1999; Minson *et al.* 2001).

Smoking a cigarette decreases resting SkBF (Roth *et al.* 1944; Eckstein *et al.* 1957; Richardson, 1987; Fushimi *et al.* 1992; Monfrecola *et al.* 1998), an effect mediated, at least in part, by an increase in central sympathetic outflow caused by nicotine (Narkiewicz *et al.* 1998). Nicotine could potentially have other effects on both components of the SkBF response to local heating. Although there is evidence that nicotinic acetylcholine receptors (nAChRs) are present on human nociceptors (Douglas & Ritchie, 1960; Parkhouse & Le Quesne, 1988; Dessirier *et al.* 1998), their physiological role is unknown. Some animal experiments suggest that stimulation of the nicotine receptors facilitates nociceptor axon reflexes (Grunfeld *et al.* 1991, 1993), which could increase SkBF. On the other hand, prejunctional nAChRs facilitate noradrenaline (norepinephrine) release from sympathetic postganglionic nerves, a factor that could inhibit SkBF responses to local warming (Starke, 1977; Kristufek *et al.* 1999). Nicotine may also affect the production of and

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responses to NO (Hashimoto, 1994; Sarabi & Lind, 2000; Gaenzer *et al.* 2001). The effects of smoking on SkBF responses to local heating are unknown. Such information is of clinical relevance because smoking is known to impair postoperative wound healing (Moller *et al.* 2002; Sorensen *et al.* 2003), which is affected by local SkBF and resulting tissue oxygenation.

With this information as a background, we tested the hypothesis that in habitual smokers deprived of nicotine for at least 12 h, acute exposure to nicotine delivered via cigarette smoke would inhibit both the initial neural component and the later sustained component of the SkBF response to local heating of human non-glabrous skin.

Methods

Subjects

This study was conducted in accordance with the standards in the *Declaration of Helsinki*, and was approved by the Mayo Foundation Institutional Review Board. Written informed consent was obtained from each subject.

The 11 subjects studied (9 female, 2 male) were all current smokers, defined as having smoked for at least 5 years, with an average daily cigarette consumption of > 10 over the last 6 months. Subjects were excluded if obese (body mass index $> 30\ \mathrm{kg\ m^{-2}}$), hypertensive, or had a history of heart disease, diabetes, or autonomic disorders. Those taking serotonin-reuptake inhibitors were also excluded. Subjects abstained from the use of non-steroidal anti-inflammatory agents for 24 h before each study, and aspirin for 1 week before the study. All subjects abstained from smoking for at least 12 h before each study, as confirmed by exhaled carbon monoxide sampling in the morning of the study. All female subjects were studied in the early follicular phase of the menstrual cycle to control for variability due to reproductive hormone status (Charkoudian *et al.* 1999). All studies began in the morning, and subjects were seated throughout the session. Each subject was scheduled to participate in two experimental sessions separated by at least 24 h.

Instrumentation

Arterial blood pressure was measured by an oscillometric automated cuff (Agilent M1204R) providing mean arterial pressure. An intravenous catheter was inserted into an antecubital vein for blood sampling to measure plasma nicotine concentrations.

Skin blood flow was measured by laser-Doppler flowmetry (LDF) (Perimed Periflux System 5000, Stockholm, Sweden) at sites on the anterior aspect of the left forearm. The laser-Doppler probes were placed in the centre of a specialized probe holder that both measured and controlled local temperature over $7\ \mathrm{cm^2}$ of the skin surface (Peritemp 4005, Stockholm, Sweden). After placement, probes were not moved until the end of the experimental session.

Based on the results of the first three subjects, in the last eight subjects bretylium was administered by iontophoresis to inhibit cutaneous adrenergic nerve transmission (Kellogg *et al.* 1989). A plastic iontophoresis chamber (Mayo Clinic Section of Engineering) similar to that described by Kellogg and colleagues (Kellogg *et al.* 1989) was attached to the forearm and filled with a 10 mM solution of bretylium tosylate dissolved in propylene glycol. A current of 250 μA was applied for 10 min, providing a current density of $400\ \mu\mathrm{A\ cm^{-2}}$. This regimen has been shown to provide complete blockade of adrenergic neurotransmission for up to 6 h (Pergola *et al.* 1996). At least 1 h elapsed between iontophoresis and LDF measurements to allow for the bretylium effect and the resolution of current-induced hyperaemia.

Local heating regimens

LDF was measured in at least two sites in all subjects. At one site, local heating was performed by increasing the set heating element temperature from a baseline of 32°C to 42°C in 0.5°C increments every 45 s (i.e. over 15 min), referred to hereafter as the RAMP protocol. At the other site, local heating was performed by increasing the set heating element temperature from a baseline of 32°C to 42°C in 0.5°C increments every 4.5 s (i.e. over 90 s), referred to hereafter as the STEP protocol (Minson *et al.* 2001). As shown in prior work, this rate of temperature change is not perceived as painful (Kellogg *et al.* 1999; Minson *et al.* 2001). For both heating protocols, heating element temperature was maintained at 42°C for 30 min. In previous work using similar experimental equipment, it was shown that LDF measurements at the conclusion of the STEP protocol are approximately 90% of the maximal values produced by intradermal microdialysis of 50 mM sodium nitroprusside (Minson *et al.* 2001), and that local skin temperature, as measured directly by thermocouples, is approximately 40°C under these conditions. The temperatures reported hereafter refer to those of the heating elements, which were consistent across conditions.

In the last eight subjects, LDF was measured at a third site pretreated with bretylium to investigate the possible involvement of noradrenergic neurotransmission in the responses. The STEP heating protocol was applied to this site.

Protocol

Following instrumentation, a 10 min baseline recording was obtained with the heating element temperature

maintained at 32°C. Heating protocols were then applied to sites as described above. Following this initial measurement of responses (requiring a total of approximately 50 min), heating element temperature was returned to 32°C, and 1 h allowed for recovery. At the end of this recovery period, SkBF measurements had consistently returned to their pre-heating values.

After this recovery period, the subjects received nicotine. On the first experimental day, they smoked their usual brand of cigarette, taking one puff every minute (requiring approximately 8 min). Thirty minutes after beginning the first cigarette, they smoked a second cigarette in the same fashion. This regimen was designed using published pharmacokinetic data to provide a relatively sustained plasma nicotine concentration over the following hour (Gourlay & Benowitz, 1997). On the second experimental day, they received 2 mg of intranasal nicotine spray (Nicotrol NS, Parmacia Consumer Heathcare), followed 30 min later by a second dose of 2 mg. This method of administration was chosen to achieve approximately the same plasma nicotine concentration as produced by cigarette smoking (Gourlay & Benowitz, 1997). The nasal spray produced transient mucosal irritation that resolved within 10 min. On both days, the response of SkBF to the administration of nicotine via cigarette smoke or nasal spray was noted with heating element temperature maintained at 32°C.

Approximately 20 min after the initiation of the second cigarette or the second intranasal nicotine administration, the heating protocols were repeated. Thus, approximately 110 min elapsed from the completion of the first heating of a site to the commencement of the second heating. At the conclusion of the second heating protocol, venous blood samples were obtained to measure plasma nicotine concentrations by isotope dilution gas chromatography–mass spectrometry (Baskin *et al.* 1998). The lower level of detection for this assay is 2 ng ml^{-1}.

We have previously shown that neither the initial peak vasodilatation nor the maximal response to sustained local heating change when the STEP heating protocol is repeated at least 1 h apart in the absence of interventions in normal subjects (Charkoudian *et al.* 2002). To evaluate whether this is also true in smokers and with the RAMP heating protocol, in three subjects nicotine was not administered between the two heating protocols on the first experimental day. Thus, in these three subjects the first day provided control data for the effect of repeated heating. On the second experimental day, they received nicotine nasal spray as described above.

Data analysis

LDF was divided by mean arterial pressure to derive an index of cutaneous vascular conductance (CVC), averaged at 1 s intervals. The reference CVC for each site was defined for this study as the mean CVC over the last 5 min of the initial (before nicotine) heating protocol (either STEP or RAMP). CVC values were expressed as a percentage of the reference value for that site.

Responses to RAMP heating were quantified by averaging CVC and heating element temperature for each individual run over 1 min intervals. A sigmoidal curve was then fitted to the relationship between temperature and CVC for each subject (Sigma Plot). In each case the correlation coefficient exceeded 0.992. Parameters from this sigmoidal relationship were then used to determine CVC at intervals of 1°C between 32 and 42°C for each subject, and to determine the temperature (T_{50}) at which CVC reached half of the difference between the values at 32 and 42°C. The difference between CVC at 32 and 42°C was also noted.

The initial phase of the response to STEP heating was quantified as the initial peak of the CVC response, which occurred within 4–6 min after the onset of local warming.

Data are presented as means ± S.D. Paired comparisons were performed using the signed rank test, as the assumptions of the paired t test were not satisfied for all data. $P < 0.05$ was taken to be statistically significant.

Results

All 11 subjects completed the first experimental day. On this first day, 8 subjects received nicotine delivered via cigarette smoking, and 3 received repeated heating without nicotine administration. Of the 8 subjects who smoked on the first experimental day, one subject elected not to complete the second experimental day (nicotine delivered via nasal spray), and data from the second day of another subject were lost due to equipment failure. Thus, data concerning nicotine effects are reported from 8 subjects who received nicotine via cigarette smoke on the first experimental day, and 9 subjects who received nicotine via nasal spray on the second experimental day (of these, 6 subjects received nicotine via both routes on these two separate days).

The plasma nicotine concentration produced by smoking two cigarettes (9.1 ± 2.1 ng ml^{-1}) was not significantly different from that produced by the two administrations of nicotine nasal spray (8.2 ± 2.2 ng ml^{-1}).

Administration of nicotine via a single cigarette or set of nasal sprays significantly increased mean arterial pressure (from 82 ± 8 to 88 ± 8 mmHg and from 84 ± 8 to 91 ± 6 mmHg for cigarettes and nasal sprays, respectively, $P < 0.01$ for each). There was no significant difference in the increases produced by the two modes of nicotine administration. The second administration

of nicotine had no further effect on MAP (data not shown).

Resting SkBF

Administration of nicotine via a single cigarette or set of nasal sprays decreased resting SkBF measured at a heating element temperature of 32°C ($P < 0.008$ for both, Fig. 1). The second administration of nicotine had no further effect on SkBF. There was no significant difference in the decreases produced by the two modes of nicotine administration. Pretreatment of the skin with bretylium significantly inhibited this decrease ($P < 0.04$ for each, Fig. 1).

RAMP heating protocol

Increasing heating element temperature from 32 to 42°C over 15 min increased SkBF (Fig. 2). Nicotine, whether administered via cigarettes or nasal spray, significantly affected this response. At lower temperatures, SkBF was significantly decreased after nicotine, consistent with the effect observed on baseline SkBF. At the midrange of temperatures, SkBF was similar before and after nicotine administration. At the highest temperature (42°C), SkBF (measured over the first minute after achieving this temperature) was greater after nicotine administration ($P = 0.008$ for cigarettes and $P = 0.039$ for nasal spray).

For both cigarette smoking and nasal spray, the difference between SkBF measured at 42 and 32°C was greater after nicotine (62 ± 13 *versus* 72 ± 9% of maximal CVC before and after cigarettes, respectively, $P = 0.008$, and 58 ± 14 *versus* 73 ± 16% of reference CVC before and after nasal spray, respectively, $P = 0.004$). The temperature (T_{50}) at which CVC reached half of the difference between the values at 32 and 42°C was not significantly affected by nicotine (39.4 ± 0.7 *versus* 39.5 ± 0.9°C before and after cigarettes, respectively, and 39.4 ± 0.6 *versus* 39.4 ± 0.7°C before and after nasal spray, respectively). The plateau CVC measured 30 min after achieving 42°C was significantly decreased after nicotine administration (to 89 ± 10% of reference CVC after cigarettes, $P = 0.008$, and 82 ± 11% of reference CVC after nasal spray, $P = 0.004$).

The temperature at which warmth was first perceived during RAMP heating tended to be less after nicotine administration, a difference that was significant after nasal spray (36.4 ± 2.4 *versus* 35.4 ± 2.0°C before and after cigarettes, respectively, $P = 0.078$, and 36.9 ± 2.2 *versus*

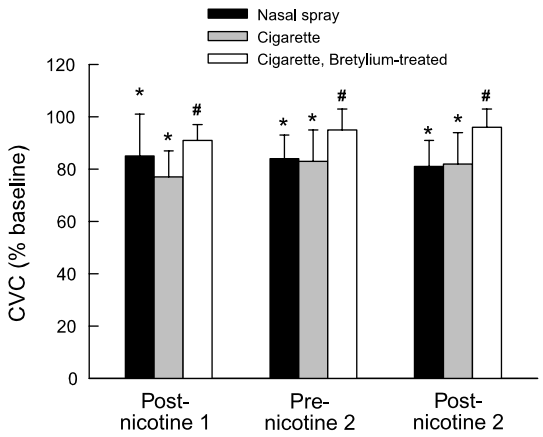

Figure 1
Changes in cutaneous vascular conductance (CVC) during two administrations of nicotine either via cigarettes ($n = 8$ experiments) or nasal sprays ($n = 9$). In some subjects, one site was treated with bretylium before cigarette smoking ($n = 5$). Values are means ± S.D. and are expressed as a percentage of baseline measured before the first administration of nicotine. * Significant difference from baseline, signed-rank test, $P < 0.05$. # Significant difference from site not treated with bretylium, signed-rank test, $P < 0.05$.

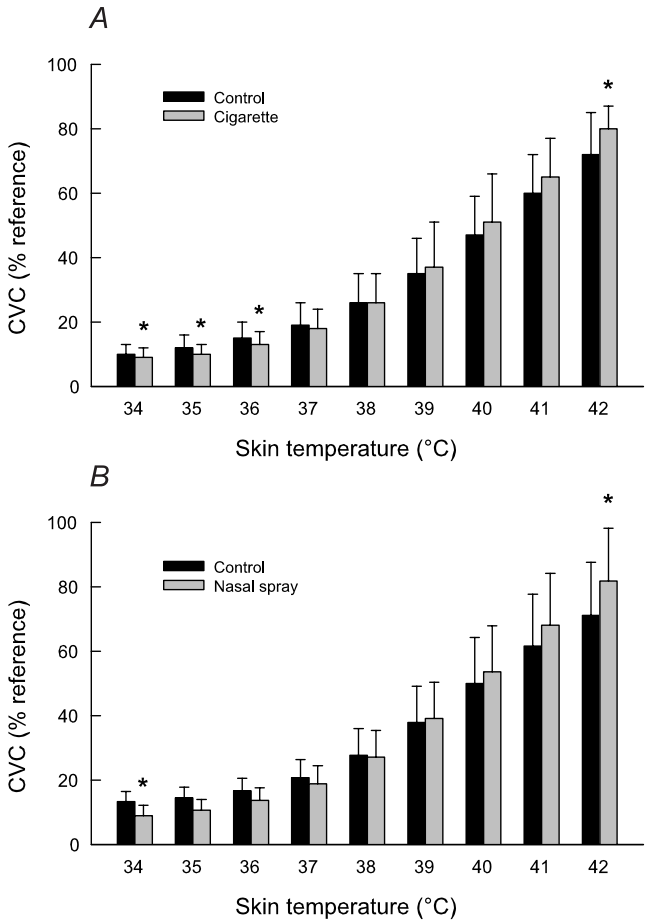

Figure 2
Cutaneous vascular conductance (CVC) as a function of local skin temperature during gradual RAMP heating (over 15 min), before (Control) and after nicotine given via cigarettes (*A*, $n = 8$) or nasal spray (*B*, $n = 9$). Values are means ± S.D. and are expressed as a percentage of the reference CVC for that site. * Significant difference from control, signed-rank test, $P < 0.05$.

35.5 ± 1.1°C before and after nasal spray, respectively, $P = 0.039$).

STEP heating protocol

Increasing the heating element temperature from 32 to 42°C over 90 s produced a biphasic increase in SkBF, characterized by a rapid increase to a peak value, then a moderate decrease, followed by a slower phase vasodilatation that attained a plateau. Figures 3 and 4 show a representative example of SkBF responses before and after cigarettes and nasal spray, respectively, and Fig. 5 shows mean baseline, peak and plateau SkBF values for all subjects. Nicotine, whether administered via cigarettes or nasal spray, significantly increased the initial peak value, and significantly decreased the plateau value. In skin pretreated with bretylium, nicotine still increased the initial peak value, but had no effect on the plateau value (Figs 3–5).

Effect of repeated heating in the absence of nicotine

Repeated local STEP or RAMP heating protocols, separated by 110 min with no other intervention, produced similar vasodilator responses in three subjects (Table 1). Thus, the repetition of heating in the absence of nicotine did not affect SkBF responsiveness.

Discussion

The major new finding of this study is that in habitual smokers abstinent from cigarettes for at least 12 h, nicotine augmented initial cutaneous vasodilator responses (measured at a heating element temperature of 42°C) to both gradual and rapid warming of non-glabrous skin by mechanisms not dependent on noradrenergic nerves. In contrast, nicotine inhibited the sustained vasodilatation produced by local heating (i.e. the response after 30 min at a heating element temperature of 42°C) by a mechanism dependent on local noradrenergic neurotransmission.

The observed reduction in resting SkBF (measured at 32°C) caused by cigarette smoking is consistent with many prior studies (Roth *et al.* 1944; Eckstein *et al.* 1957; Richardson, 1987; Fushimi *et al.* 1992; Monfrecola *et al.* 1998). Most suggest that vasoconstriction is caused primarily by nicotine rather than other components of smoke (Maddock & Coller, 1932; Weatherby, 1942; Roth

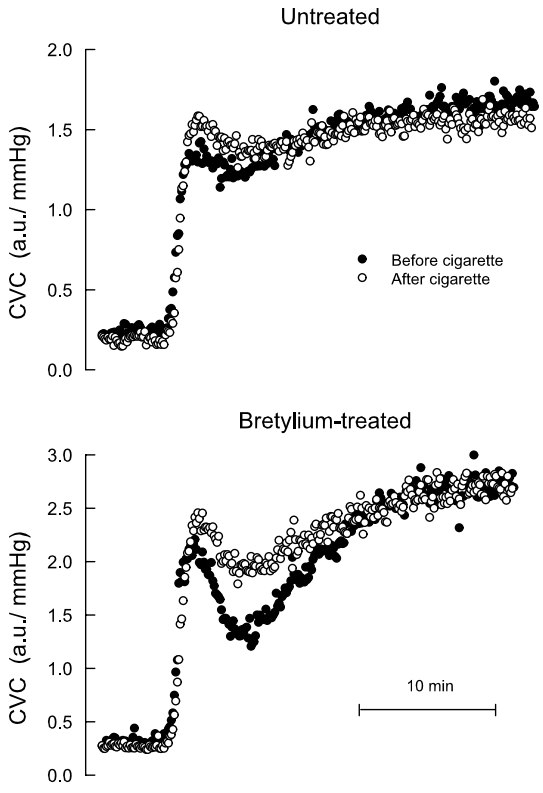

Figure 3
Cutaneous vascular conductance (CVC) over time during rapid STEP heating in one subject before (●) and after (○) cigarette smoking, measured at separate sites not treated and treated with bretylium delivered via iontophoresis.

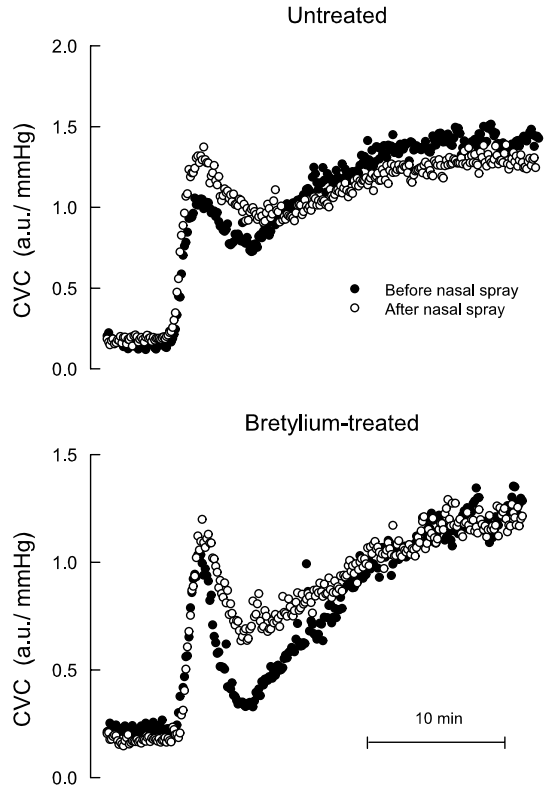

Figure 4
Cutaneous vascular conductance (CVC) over time during rapid STEP heating in one subject (the same subject whose data are depicted in Fig. 3) before (●) and after (○) nicotine nasal spray, measured at separate sites not treated and treated with bretylium delivered via iontophoresis.

et al. 1944; Fushimi *et al.* 1992), which is consistent with our results, although some studies show that smoking cigarettes with very low nicotine can also reduce blood flow (Evans & Stewart, 1943; Richardson, 1987). Circulating catecholamines do not appear to contribute significantly to this vasoconstriction (Coffman, 1967; Cryer *et al.* 1976). Rather, smoking increases sympathetic outflow to the skin (Narkiewicz *et al.* 1998). In addition, prejunctional nAChRs may enhance noradrenaline release from postganglionic sympathetic neurones (Miao *et al.* 1996; Kristufek *et al.* 1999; Si & Lee, 2002). The ability of bretylium to attenuate nicotine-induced decreases in resting SkBF is consistent with nicotine-induced enhancement of local noradrenaline release via either or both of these mechanisms (Cryer *et al.* 1976; Grassi *et al.* 1994).

Nicotine also attenuated the sustained vasodilatation produced by local heating (measured 30 min after achieving a probe temperature of 42°C). This sustained vasodilator response is mediated at least in part by nitric oxide, as it is inhibited by preventing NO formation in the skin (Kellogg *et al.* 1999; Minson *et al.* 2001). The finding that bretylium blocked nicotine-induced attenuation of the sustained response suggests that this attenuation was mediated by enhanced noradrenergic neurotransmission rather than by the inhibition of endothelial NO release, a postulated mechanism in other vascular beds (Hashimoto, 1994; Habler *et al.* 1999; Sarabi & Lind, 2000). The ability of sympathetic activity to modulate vascular axon reflex responses in skin is established (Hornyak *et al.* 1990; Habler *et al.* 1997). However, Pergola *et al.* (1993) found that increased sympathetic activation produced by whole-body cooling did not affect SkBF responses of human forearm skin to a local temperature of 42°C. It is possible that the ability of nicotine to reduce SkBF under similar conditions reflects the ability of prejunctional nAChRs to increase noradrenaline release, as discussed above.

In contrast to these reductions in resting SkBF and the sustained SkBF response to local heating, nicotine augmented the initial phase of SkBF responses to local non-painful heating from 32 to 42°C, whether this increase in temperature occurred over 90 s (STEP heating) or 15 min (RAMP heating). The mechanism producing this effect did not involve noradrenergic neurotransmission, as it was still present after bretylium pretreatment. For at least the more rapid heating protocol, the initial increase in SkBF is mediated by an axon reflex (Minson *et al.* 2001). The observed effect of nicotine thus implies that it modulated this reflex, which is mediated by primary nociceptive afferents (Holzer, 1992). These afferents can respond to noxious mechanical stimulation (causing the perception of pain), chemical, cold, and heat stimuli. The neural elements transducing temperature-regulated mediator release are members of the transient receptor potential (TRPV) channel subfamily that include the vanilloid receptor (VR1) activated by capsaicin, as well as other capsaicin-insensitive receptors that may mediate responses to higher temperatures (Caterina *et al.* 1997; Zygmunt *et al.* 1999; Schwarz *et al.* 2000; Xu *et al.* 2002). Receptor stimulation causes vasodilatation (Parkhouse & Le Quesne, 1988; Wardell *et al.* 1993; Magerl & Treede, 1996; Stephens *et al.* 2001) via the release of neuropeptides such as CGRP and pituitary adenylate cyclase-activating polypeptide (PACAP), which coexists with CGRP in capsaicin-sensitive afferents (Mulder *et al.* 1994; Zygmunt *et al.* 1999). Both compounds produce cutaneous vasodilatation in humans (Dorner *et al.* 1998) and may

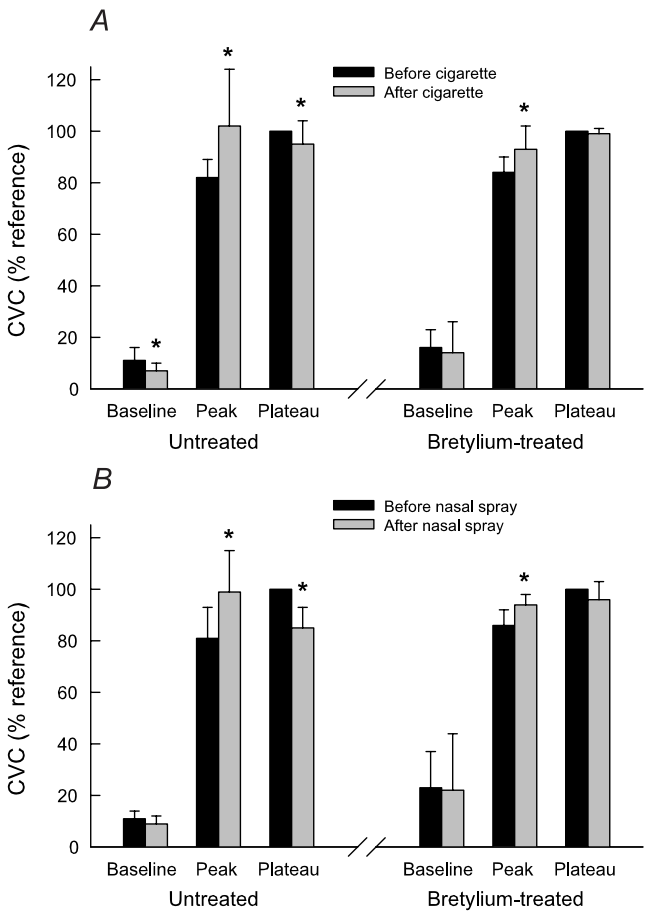

Figure 5
Cutaneous vascular conductance (CVC) measured at three times during rapid STEP heating: at baseline before heating (averaged over the 5 min prior to heating), at the peak of the initial response, and at a plateau of the sustained response after 30 min maintained at 42°C (averaged over the last 5 min of this period). The effect of cigarette smoking on these values is shown in *A*, and that of nicotine nasal spray in *B*, both in separate sites not treated ($n = 8$ for cigarettes and $n = 9$ for nasal spray) and treated with bretylium via iontophoresis ($n = 5$ for cigarettes and $n = 6$ for nasal spray). Values are means ± S.D. and are expressed as a percentage of the reference CVC for that site. * Significant difference from before nicotine administration, signed-rank test, $P < 0.05$.

Table 1. Responses to repeated heating in the absence of nicotine

	Response to first heating (%ref CVC)	Response to second heating (%ref CVC)
RAMP heating (°C)		
34	9 ± 2	6 ± 1
35	11 ± 3	8 ± 1
36	16 ± 4	13 ± 2
37	23 ± 7	20 ± 4
38	34 ± 10	33 ± 8
39	48 ± 14	49 ± 13
40	62 ± 17	64 ± 16
41	75 ± 17	77 ± 17
42	83 ± 16	85 ± 15
STEP heating		
Untreated		
Peak	91 ± 17	89 ± 19
Plateau	100	95 ± 17
Bretylium-treated		
Peak	84 ± 6	84 ± 4
Plateau	100	93 ± 8

Responses (% reference CVC) were determined at three sites: one exposed to RAMP heating (see text for description), one exposed to STEP heating, and a third exposed to STEP heating after bretylium iontophoresis. CVC: cutaneous vascular conductance. Values are means ± S.D. from three subjects.

regulate other key elements of the response to local tissue trauma (Schaffer *et al.* 1998; Smith & Liu, 2002).

Application of acetylcholine or nicotine to tissues produces pain and inflammation (Dessirier *et al.* 1998). Exploration of this observation has revealed the presence of nAChRs on primary nociceptors in several tissues, including skin (Douglas & Ritchie, 1960; Steen & Reeh, 1993; Dessirier *et al.* 1998; Jinks & Carstens, 1999). Activation of these receptors, which are found on both capsaicin-sensitive and -insensitive afferents (Roberts *et al.* 1995), can produce the release of neuropeptides (Jinno *et al.* 1994; Puttfarcken *et al.* 1997), vasodilatation (Grunfeld *et al.* 1991), and plasma extravasation (Miao *et al.* 2001). The latter effect can occur at subnanomolar local nicotine concentrations in animal experiments. Although their physiological function is obscure, many tegmental cells (including skin keratinocytes) synthesize and secrete acetylcholine (Conti-Fine *et al.* 2000), which may act in a paracrine fashion to mediate responses to tissue injury.

Based on this information and our present results, we propose that the stimulation of nAChRs on nociceptive afferents following smoking or nasal spray enhances the release of vasodilating neuropeptides from these fibres in response to stimulation of TRPV-subfamily receptors by heat. Such an effect would be analogous to the function of presynaptic nAChRs that modulate the release of neurotransmitters in the central nervous system (Wonnacott, 1997). Support for this mechanism is provided by the observations of Bernardini *et al.* (2001*a*,*b*). Using an isolated saphenous nerve–skin preparation from the rat, they found that nicotine excited nociceptive afferents and caused the release of CGRP. Furthermore, nicotine also augmented heat-induced increases in afferent activity in a dose-dependent manner. Chronic exposure of rats to systemic nicotine causes enhanced SkBF responses to iontophoresed acetylcholine, providing support for the concept that systemic nicotine can also modulate axon reflexes *in vivo* (Grunfeld *et al.* 1991, 1993).

In human non-glabrous skin, iontophoresis of acetylcholine also produces cutaneous vasodilatation mediated at least in part by an axon reflex (Parkhouse & Le Quesne, 1988; Berghoff *et al.* 2002). However, this vasodilatation can be blocked by atropine (Kellogg *et al.* 1995), suggesting involvement of muscarinic rather than nicotinic mechanisms in responses to high local concentrations of acetylcholine. The activation of nicotinic receptors in skin appears to be facilitatory rather than a primary stimulus for vasodilatation, at least for the degree of stimulation produced by systemic nicotine. Indeed, in the present study nicotine produced vasoconstriction in the absence of heating, suggesting either that stimulation by systemic nicotine was insufficient to trigger axon reflexes in the absence of heating, or that increased noradrenaline release produced by nicotine overcame any reflex vasodilatation.

Sensitization of nociceptive afferents is also suggested by the finding that nicotine tended to decrease the temperature at which warmth was first perceived during gradual heating. This interpretation assumes that perception is related to afferent activity, such that stimulation of nicotine receptors reduced the heat for TRPV receptor activation to reach perception threshold. A similar phenomenon has been observed when capsaicin was used to stimulate nociceptors in the skin, producing a decrease in the temperature of initial warmth perception during heating (Stephens *et al.* 2001). However, unlike that previous study we did not observe a shift in the position of the curve describing the relationship between skin temperature and SkBF. This may be related to the effect nicotine has of reducing baseline SkBF, making the interpretation of T_{50} in these terms problematic.

Although our findings are consistent with our proposed mechanism, given the ubiquity of nicotinic receptors in other systems responsible for SkBF regulation (e.g. autonomic ganglia) and the complex regulation of SkBF during heating, other mechanisms cannot be excluded. Further studies will be necessary to confirm that direct nicotinic enhancement of axon reflexes is the salient mechanism for the observed augmentation of initial SkBF responses to local heating.

It is also important to note that these findings may be specific to chronic smokers. Exposure to nicotine produces both acute and chronic tolerance to its effects that is specific to the response examined (e.g. psychological effects *versus* cardiovascular effects) (Perkins *et al.* 1994). Acute tolerance may develop within minutes, and resolve within hours (Porchet *et al.* 1988). Chronic tolerance requires years to develop, and may not change even after years of abstinence (Perkins *et al.* 2001). In animal models, tolerance is generally associated with an increase in central nAChR number but a decrease in function (Marks *et al.* 1993). There is no information regarding how chronic nicotine exposure affects peripheral nAChRs. Thus, our results must be interpreted in the context of possible chronic changes in nAChR function. For example, if the function of nAChRs on nociceptive afferents is decreased in habitual smokers, the increased peak vasodilatation may simply represent a restoration of a more normal response. On the other hand, the increased response may be indicative of an upregulation of peripheral nAChR function ('sensitization') caused by chronic nicotine exposure. Thus, nicotine effects on SkBF may differ in non-smoking subjects. However, such studies may prove challenging, as in naive subjects nicotine produces symptoms of anxiety, nausea, and dysphoria (Heishman & Henningfield, 2000), additional central actions which may themselves affect autonomic function and skin blood flow responses.

In summary, we observed that acute exposure to nicotine alters locally mediated vasodilatation in the skin of chronic smokers. The results suggest that peripheral nAChRs on primary nociceptive afferents in humans chronically exposed to nicotine can modulate the release of vasodilator neuropeptides that mediate the initial SkBF response to local heating. Nicotine also affects SkBF responses by causing the release of noradrenaline from noradrenergic nerves. These effects of nicotine on skin blood flow may have important implications for processes such as wound healing, in which local changes in skin blood flow are essential for normal function.

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