Nitric oxide inhibits cutaneous vasoconstriction to exogenous norepinephrine

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Shibasaki M, Low DA, Davis SL, Crandall CG. Nitric oxide inhibits cutaneous vasoconstriction to exogenous norepinephrine. J Appl Physiol 105: 1504–1508, 2008. First published September 18, 2008; doi:10.1152/japplphysiol.91017.2008.—Previously, we found that nitric oxide (NO) inhibits cutaneous vasconstrictor responsiveness evoked by whole body cooling, as well as an orthostatic stress in the heat-stressed human (Shibasaki M, Durand S, Davis SL, Cui J, Low DA, Keller DM, Crandall CG. J Physiol 585: 627–634, 2007). However, it remains unknown whether this response occurs via NO acting through presynaptic or postsynaptic mechanisms. The aim of this study was to test the hypothesis that NO is capable of impairing cutaneous vasoconstriction via postsynaptic mechanisms. Skin blood flow was monitored over two forearm sites where intradermal microdialysis membranes were previously placed. Skin blood flow was elevated four- to fivefold through perfusion of the NO donor sodium nitroprusside at one site and through perfusion of adenosine (primarily non-NO mechanisms) at a second site. Once a plateau in vasodilation was evident, increasing concentrations of norepinephrine (1 × 10−8 to 1 × 10−2 M) were administrated through both microdialysis probes, while the aforementioned vasodilator agents continued to be perfused. Cutaneous vascular conductance was calculated by dividing skin blood flow by mean arterial blood pressure. The administration of norepinephrine decreased cutaneous vascular conductance at both sites. However, the dose of norepinephrine at the onset of vasoconstriction (−5.9 ± 1.3 vs. −7.2 ± 0.7 log M norepinephrine, P = 0.021) and the concentration of norepinephrine resulting in 50% of the maximal vasocostrictor response (−4.9 ± 1.2 vs. −6.1 ± 0.2 log M norepinephrine dose; P = 0.012) occurred at significantly higher norepinephrine concentrations for the sodium nitroprusside site relative to the adenosine site, respectively. These results suggested that NO is capable of attenuating cutaneous vasocostrictor responsiveness via NOdependent mechanisms.

 METHODS

Nine healthy subjects (3 men and 6 women) participated in this study. Their mean ± SD age, height, and weight were 32.2 ± 5.8 yr, 172.4 ± 8.8 cm, and 69.3 ± 5.8 kg, respectively. The phase of the menstrual cycle was not controlled in this study. Each subject was informed of the purpose and risks of this study before providing his or her written consent. The consent form and protocol were approved by Internal Review Boards at the University of Texas Southwestern Medical Center at Dallas and at Presbyterian Hospital of Dallas.

Measurements. Upon entering the laboratory (ambient temperature: 24–25°C), subjects were instrumented for the measurement of electrocardiogram (Agilent) and arterial blood pressure via auscultation of the brachial artery (Suntech). Subjects were placed in the supine position on a patient bed. Heart rate was continuously obtained from the electrocardiogram with the signal interfaced with a cardioichometer (CWE). Mean arterial pressure was calculated as one-third pulse pressure while individuals are in this thermal condition. In heat-stressed individuals, the reduction in cutaneous vascular conductance (CVC) during a hypotensive challenge primarily occurs via withdrawal of active vasodilator activity (17). However, we recently identified that the cutaneous vasoconstrictor system can be engaged if the active vasodilator system is blocked (30, 31). This has led to the hypothesis that substances released in association with cutaneous active vasodilatation may inhibit cutaneous vasoconstriction. One such substance may be nitric oxide (NO), given that NO has been implicated in inhibiting vasoconstrictor responses in animal (2, 3, 9, 10, 19) and human (5, 6, 11, 30, 31), coupled with the recognition that a component of cutaneous vasodilation during heat stress is NO dependent (16, 18). Consistent with this hypothesis, in human skin, we and others have shown that exogenous and endogenous NO has the capacity to inhibit sympathetically mediated vasoconstriction (7, 11, 31), although this is not consistently observed (20).

The mechanism(s) through which NO attenuates cutaneous vasoconstriction remains unknown. Possibilities include presynaptic inhibition of norepinephrine release from sympathetic vasoconstrictor nerves, reduced bioactivity of norepinephrine, and/or inhibition of α-adrenergic receptor responsiveness to norepinephrine, each of which has been shown to occur in noncutaneous tissue (2, 3, 9, 10, 19, 36). Thus it remains unknown whether NO-dependent attenuation of cutaneous vasoconstrictor responsiveness is caused by presynaptic inhibition and/or postsynaptic inhibition. This study specifically examined the hypothesis that NO is capable of attenuating cutaneous vasoconstrictor responsiveness via postsynaptic mechanisms.

IN HUMANS, SKIN BLOOD FLOW (SKBF) is controlled by a sympathetic adrenergic system, primarily responsible for reductions in SKBF during conditions such as whole body cold stress, and by a separate active vasodilator system, which mediates 90–95% of the increase in SKBF during a heat stress (13, 15). Classically, it has been thought that these systems are functionally separate. However, recent evidence suggests that the responsiveness of the sympathetic adrenergic system can be modulated by factors associated with the cutaneous active vasodilator system (30, 31).

During pronounced heat stress, upwards to ~50% of cardiac output is distributed to the cutaneous circulation (25, 27); thus the control of SKBF becomes important for blood pressure regulation while individuals are in this thermal condition. In heat-stressed individuals, the reduction in cutaneous vascular conductance (CVC) during a hypotensive challenge primarily occurs via withdrawal of active vasodilator activity (17). However, we recently identified that the cutaneous vasoconstrictor system can be engaged if the active vasodilator system is blocked (30, 31). This has led to the hypothesis that substances released in association with cutaneous active vasodilatation may inhibit cutaneous vasoconstriction. One such substance may be nitric oxide (NO), given that NO has been implicated in inhibiting vasoconstrictor responses in animal (2, 3, 9, 10, 19) and human (5, 6, 11, 30, 31), coupled with the recognition that a component of cutaneous vasodilation during heat stress is NO dependent (16, 18). Consistent with this hypothesis, in human skin, we and others have shown that exogenous and endogenous NO has the capacity to inhibit sympathetically mediated vasoconstriction (7, 11, 31), although this is not consistently observed (20).

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pressure plus diastolic pressure. Two microdialysis probes were placed in the intradermal space of the dorsal aspect of one forearm. SkBF was indexed directly over each microdialysis membrane using multifiber laser Doppler flowmetry (Perimed).

**Protocol.** Approximately 90–120 min after probe placement, once the hyperemic response associated with membrane placement subsided, the following procedures were performed. One probe was perfused with 0.025 to 2.5 mM of the NO donor sodium nitroprusside (SNP), and the other probe was perfused with $5.6 \times 10^{-3}$ to $5.6 \times 10^{-10}$ M adenosine, both at a rate of 2 $\mu$L/min. The goal of these pharmacological manipulations was to increase SkBF at both sites equally (i.e., 5-fold) relative to preinfusion levels, but via NO (SNP) and primarily non-NO (adenosine) mechanisms. Once a plateau in vasodilation was evident (i.e., minimum of ~15 min), increasing concentrations of norepinephrine ($10^{-8}$ to $10^{-2}$ M) in the final concentration of SNP or adenosine (i.e., the final concentration of SNP and adenosine were kept constant throughout) were administrated through both microdialysis probes, with each norepinephrine dose being administered for 5 min (see Fig. 1).

**Data analysis.** Data were recorded at 50 Hz via a 16-bit analog-to-digital converter (Biopac, Santa Barbara, CA). CVC was calculated from the ratio of SkBF to mean arterial blood pressure and normalized to the peak CVC after SNP and adenosine administration, but before the first dose of norepinephrine. Absolute CVC, expressed as arbitrary units (arbitrary units/mmHg), was also analyzed. Average CVC during the final 30 s of each dose of norepinephrine was obtained and compared across sites and doses of norepinephrine via a repeated-measures two-way ANOVA. Mathematical modeling was used to identify the effective drug concentration causing 50% of the maximal vasoconstrictor response (i.e., EC$_{50}$). An important component of the analysis was the identification of the dose of norepinephrine that resulted in a sustained reduction in CVC. Because of variability in CVC, these data were extracted and averaged across 30-s windows during the final 2 min of SNP and adenosine administration, but before norepinephrine administration. These data were then converted into a percentage of peak vasodilation achieved with adenosine or SNP. The maximum average inherent oscillation of CVC was 2.6%.

Mean arterial blood pressure and heart rate did not change throughout the protocol. Baseline CVC before the administration of SNP and adenosine was similar between sites (Table 1). SNP and adenosine significantly increased CVC at both sites $\sim 5.5$-fold above predrug baseline (SNP: $5.8 \pm 2.9$-fold, adenosine: $5.5 \pm 2.7$-fold), but the magnitude of these increases was not different between sites (Table 1).

Administration of norepinephrine significantly decreased CVC at both sites (Fig. 2). The ANOVA revealed a significant interaction, suggesting that the vasoconstricting effect of norepinephrine was influenced by the administered vasodilating substance. The threshold dose of norepinephrine resulting in the onset of the sustained reduction in CVC was greater at the SNP site ($5.9 \pm 1.3$ log M norepinephrine) relative to the adenosine site ($7.2 \pm 0.7$ log M norepinephrine, $P = 0.021$). Consistent with this finding, the EC$_{50}$ of the dose-response curve was higher for the SNP site ($4.9 \pm 1.2$ log M norepinephrine) relative to the adenosine site ($6.1 \pm 0.2$ log M norepinephrine, $P = 0.012$). At the highest dose of norepinephrine, CVC was significantly greater at the SNP site relative to the adenosine site. Mean CVC at the adenosine site significantly decreased below the preinfusion baseline at the highest dose of norepinephrine, with each subject exhibiting such a response. In contrast, at the highest dose of norepinephrine, mean CVC at the SNP site was not significantly less than preinfusion baseline, given that CVC was higher relative to the preinfusion baseline in seven of nine subjects.

**DISCUSSION**

The primary objective of this study was to test the hypothesis that NO attenuates cutaneous vasoconstrictor responses to exogenous norepinephrine via postsynaptic mechanisms. To that end, the results clearly confirm this hypothesis as the...
magnitude of cutaneous vasoconstriction to norepinephrine administration at the site dilated with SNP was significantly attenuated relative to the site dilated with adenosine.

SkBF is under baroreflex control (12, 14, 26). In normothermic subjects, a hypotensive challenge decreases CVC primarily via the cutaneous sympathetic vasoconstrictor system (4, 17). In contrast, reductions in CVC during a hypotensive challenge in heat-stressed individuals are reported to occur primarily via the withdrawal of active vasodilator neural activity (17). Recently, our laboratory found that the cutaneous vasoconstrictor system can be engaged and contribute to the reduction in CVC in heat-stressed individuals during baroreceptor unloading, if the active vasodilator system is blocked (30). Furthermore, the reduction in CVC to this hypotensive challenge was significantly greater at the site where the active vasodilator system was blocked. This observation raised the hypothesis that neurotransmitters released from cutaneous active vasodilator nerves, or downstream factors evoked by those neurotransmitters, have the capability of inhibiting cutaneous vasoconstrictor responsiveness.

Although the neurotransmitter(s) responsible for cutaneous active vasodilation remains unclear, NO has been shown to contribute to this response (16, 28, 32). We and others have sought to identify whether NO is capable of attenuating cutaneous vasoconstrictor responsiveness and to identify the mechanisms of this hypothesized response. Consistent with this hypothesis, cutaneous vasoconstriction to whole body cooling was attenuated at NO-treated sites relative to sites where CVC was matched via a primarily non-NO mechanism (7, 11), indicating that exogenous NO can attenuate adrenergic-mediated cutaneous vasoconstriction. In a follow-up study, NO synthase was inhibited via local administration of N\textsuperscript{G}-nitro-L-arginine methyl ester, and likewise a greater reduction in CVC at the N\textsuperscript{G}-nitro-L-arginine methyl ester-treated site occurred during a hypotensive challenge (via lower body negative pressure) of heat-stressed individuals (31). Together, these observations strongly suggest that NO has the capability of exerting a sympatheticolytic effect in the cutaneous circulation.

In noncutaneous vascular beds, a number of mechanistic studies have shown that NO attenuates vasoconstrictor responses via attenuating norepinephrine release from adrenergic nerves (i.e., presynaptic mechanisms), as well as by reducing the effectiveness of released norepinephrine (i.e., postsynaptic mechanisms) (2, 3, 9, 10, 19, 36). Thus reduced cutaneous vasoconstrictor responsiveness to NO could be due to reduced release of norepinephrine from nerve terminals and/or reduced effectiveness of released norepinephrine. Consistent with this thought, we previously found that cutaneous vasoconstrictor responses to exogenous norepinephrine were attenuated when the skin was vasodilated secondary to increases in internal temperature or via local heating (34); both perturbations having a NO-dependent component (16, 18, 23, 28, 29, 32). This was evidenced by a significant increase in the EC\textsubscript{50} of the dose-response curves during both heating protocols relative to the EC\textsubscript{50} of the non-heat-stressed conditions. However, a limitation of that study was the difference in baseline CVC between the control and heated conditions. By providing a primarily non-NO-mediated flow control site, via adenosine, the present study found that the reduction in CVC to exogenous norepinephrine administration was significantly blunted at the site receiving the NO donor SNP compared with an adjacent site receiving adenosine. This observation supports the proposed hypothesis that NO is capable of inhibiting cutaneous vasoconstriction by attenuating the responsiveness to norepinephrine and extends the interpretation of the prior work by providing evidence that this response can occur via postsynaptic mechanisms.

Adenosine was employed to match cutaneous vasodilation that occurred with SNP administration at an adjacent site. A component of the vasodilator response to adenosine may be NO dependent (22), although adenosine still causes substantial vasodilation at sites where NO synthase has been inhibited (33). Nevertheless, we cannot exclude the possibility that some of the vasodilator response to adenosine was NO dependent. If this was the case, then it is equally a possibility that adenosine-mediated NO release had some effect at attenuating cutaneous vasoconstrictor responsiveness. Despite this possibility, cutaneous vasoconstrictor responsiveness to exogenous norepinephrine remained greater at the adenosine-treated site relative to the SNP-treated site. Depending on the contribution of NO to adenosine-mediated cutaneous vasodilation, perhaps even greater difference in vasoconstrictor responsiveness may have been observed between SNP and adenosine-treated sites to exogenous norepinephrine had NO synthesis been blocked at the adenosine-treated site. Although such a protocol may be insightful, this protocol was not necessary to test the hypothesis that NO is capable of attenuating cutaneous vasoconstrictor responsiveness to exogenous norepinephrine.

### Table 1. Cutaneous vascular conductance for the indicated vasodilator drug and condition

<table>
<thead>
<tr>
<th>Pharmacological Agent</th>
<th>Plateau Vasodilation to the Indicated Pharmacological Agent</th>
<th>CVC at the Highest Dose of NE (10\textsuperscript{-2} M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>SNP 0.27±0.09</td>
<td>1.47±0.48*</td>
</tr>
<tr>
<td>Pharmacological Agent</td>
<td>SNP 0.32±0.12</td>
<td>1.70±0.88*</td>
</tr>
</tbody>
</table>

Values are means ± SE in arbitrary units/mmHg. SNP, sodium nitroprusside; CVC, cutaneous vascular conductance; NE, norepinephrine. *Significantly different from the baseline, P < 0.05. †Significantly different between SNP and adenosine sites, P < 0.05.

![Fig. 2. Cutaneous vascular conductance (CVC) to varying concentrations of exogenous NE at SNP- and adenosine-treated sites. Data are expressed as a percentage of peak vasodilation to SNP and adenosine administration before NE administration. NE doses are depicted as log-molar concentration. *P < 0.05, SNP vs. adenosine. EC\textsubscript{50}, 50% of the maximal vasoconstrictor response.](http://jap.physiology.org/)

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Kolo et al. (19) proposed that NO may reduce the bioavailability of norepinephrine. Other studies suggest that NO can alter postsynaptic vasoconstrictor responsiveness (2, 10). Given these findings, whether the observed attenuated cutaneous vasoconstrictor responsiveness to exogenous norepinephrine at the SNP-treated site is a result of reduced bioavailability of norepinephrine or due to altered vasoconstrictor responsiveness cannot be distinguished in the present investigation. Further study is, therefore, needed to identify the precise mechanisms by which NO attenuates cutaneous vasoconstrictor responsiveness to exogenous norepinephrine administration. Moreover, a potential effect of NO in altering presynaptic release of norepinephrine was not investigated in this study. Further studies are, therefore, also warranted to identify whether NO attenuates norepinephrine release from cutaneous adrenergic nerves, as has been reported in other tissues (3, 9).

The menstrual cycle was not controlled in the present study. Numerous studies have shown that hormones associated with the menstrual cycle (primarily estrogen) can stimulate NO synthase, thereby increasing NO production (1, 8, 35, 37), although the magnitude of vasodilation to NO donors is unaffected by estrogen concentrations (21, 24). As opposed to NO generation via NO synthase, in the present study, SNP was administered as a NO donor, and thus it is unlikely that estrogen variations associated with the menstrual cycle altered NO concentrations at the sites at which SKBF was assessed. That said, the effects of the menstrual cycle on the inhibition of vasoconstriction to adrenergic agents by NO remains unknown and would warrant a study specifically investigating this question.

In conclusion, the present results support the hypothesis that, in human skin, NO is capable of modulating the responsiveness of the cutaneous vasoconstrictor system via postsynaptic mechanisms. The implications of this finding raise the possibility that cutaneous NO associated with whole body heat stress may alter postsynaptic vasoconstrictor responsiveness to exogenous norepinephrine. The implications of this finding raise the possibility that cutaneous NO associated with whole body heat stress may alter postsynaptic vasoconstrictor responsiveness to exogenous norepinephrine. Given that, during a severe heat stress, inadequate cutaneous responsiveness may contribute to compromised blood pressure control through blunted cutaneous vasocconstriction.

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REFERENCES


