Preserved reflex cutaneous vasodilation in cystic fibrosis does not include an enhanced nitric oxide-dependent mechanism

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Wilkins BW, Martin EA, Roberts SK, Joyner MJ. Preserved reflex cutaneous vasodilation in cystic fibrosis does not include an enhanced nitric oxide-dependent mechanism. J Appl Physiol 102: 2301–2306, 2007. First published April 5, 2007; doi:10.1152/japplphysiol.00013.2007.—In humans, vasoactive intestinal peptide (VIP) may play a role in reflex cutaneous vasodilation during body heating. We tested the hypothesis that the nitric oxide (NO)-dependent contribution to active vasodilation is enhanced in the skin of subjects with cystic fibrosis (CF), compensating for sparse levels of VIP. In 2 parallel protocols, microdialysis fibers were placed in the skin of 11 subjects with CF and 12 controls. Lactated Ringer ester (2.7 mg/ml) was perfused at a second microdialysis site. Skin blood flow was monitored over each site with laser-Doppler flowmetry. In protocol 1, local skin temperature was increased 0.5°C every 5 s to 42°C, and then it maintained at 42°C for ~45 min. In protocol 2, subjects wore a tube-lined suit perfused with water at 50°C, sufficient to increase oral temperature (Toral) 0.8°C. Cutaneous vascular conductance (CVC) was calculated (flux/mean arterial pressure) and scaled as percent maximal CVC (sodium nitroprusside; 8.3 mg/ml). Vasodilation to local heating was similar between groups. The change (Δ%CVCmax) in CVC with NO synthase inhibition on the peak (9 ± 3 vs. 12 ± 5%CVCmax; P = 0.6) and the plateau (45 ± 3 vs. 35 ± 5%CVCmax; P = 0.1) phase of the skin blood flow response to local heating was similar in CF subjects and controls, respectively. Reflex cutaneous vasodilation increased CVC in CF subjects (58 ± 4%CVCmax) and controls (53 ± 4%CVCmax; P = 0.37) and NO synthase inhibition attenuated CVC in subjects with CF (37 ± 6%CVCmax) and controls (35 ± 5%CVCmax; P = 0.8) to a similar degree. Thus the preservation of cutaneous active vasodilation in subjects with CF is not associated with an enhanced NO-dependent vasodilation.

The mechanisms governing thermal vasodilation in human skin are complex and likely include redundant pathways and vasoactive substances. In fact, studies have identified multiple vasodilator substances contributing to cutaneous active vasodilation with increasing core temperature in humans, including nitric oxide (NO; 4, 6, 19), histamine (22), and prostaglandins (10). Despite intensive investigation, the primary neurotransmitters initiating thermal vasodilation have not been positively identified. Investigation of a role for neuropeptides in cutaneous active vasodilation have indirectly identified vasoactive intestinal peptide (VIP; 1, 18) and substance P (21) as potential neurotransmitters for active vasodilation in human skin. Along these lines, neuropeptide content is altered in cutaneous nerves of individuals with cystic fibrosis (CF; 3, 16), providing a unique opportunity to examine cutaneous vascular control in human skin.

Very little experimental data exist related to thermal cutaneous vasodilation of individuals with CF. To our knowledge, only one published report examined cutaneous vasodilation during localized forearm heating and whole body heat stress in individuals with CF. Savage et al. (16) reported preserved cutaneous active vasodilation during body heating in four subjects with CF, despite sparse VIP immunoreactivity in cutaneous nerves. In their report, the authors suggest the preserved cutaneous active vasodilation observed in subjects with CF must be possible in the face of sparse VIP immunoreactivity. In this context, cutaneous vasodilation to exogenous VIP administration includes a substantial NO-dependent component (18) and evidence suggests that NO may interact synergistically with other vasodilator signals to elicit active vasodilation in human skin (19). These observations raise the possibility that augmented NO-dependent mechanisms may account for the preserved blood flow responses in individuals with CF. In support of this possibility, Savage et al. reported higher skin blood flow responses during localized forearm heating in subjects with CF. Because local skin heating is known to include a substantial NO-dependent component (11), cutaneous vasodilation in individuals with CF may include a generalized upregulation of NO-dependent pathways.

With this information as a background, the primary purpose of the present study was to determine the contribution of NO-dependent mechanisms to thermal vasodilation in subjects with CF. Therefore, we investigated the NO-dependent vasodilation during local skin heating and the contribution of NO-dependent mechanisms to active cutaneous vasodilation during whole body heating in subjects with CF. We hypothesized that the NO-dependent portion of the skin blood flow responses to local heating would be enhanced in subjects with CF. We further hypothesized that the preserved skin blood flow response during passive whole body heating in subjects with CF would be accomplished through an enhanced NO-dependent vasodilation.

METHODS

Subjects

Eleven subjects with CF (4 women and 7 men) and 12 control subjects (4 women and 8 men) volunteered to participate in two parallel protocols. Nine CF subjects and nine control subjects participated in each protocol. Seven subjects with CF participated in both protocols and six control subjects participated in both protocols. Subjects with CF had been previously diagnosed by a physician with...
a relevant specialty and were free of other disease complications as a result of highly disciplined adherence to a comprehensive disease management program. Subjects with CF continued to take all prescribed medications specific to their disease. Control subjects were moderately active, nonsmokers, nonobese, normotensive, and not taking any medications other than oral contraceptives. Female subjects were studied during the placebo phase of oral contraceptive use, or in the early follicular phase of their menstrual cycle, to minimize possible confounding influences of reproductive hormones on the control of blood flow (2, 12). Subjects fasted overnight and refrained from caffeine or alcohol use and exercise 24 h before the study. Written informed consent was obtained from each subject before participation in this protocol that was approved by the Institutional Review Board at the Mayo Clinic.

**Instrumentation**

Protocols were performed in a temperature controlled laboratory with the subject lying supine. Subjects were instrumented with an electrocardiogram, they were monitored continuously during each protocol, and arterial blood pressure was assessed every 5 min via automated arm cuff (CardioCap, Datex-Ohmeda, Tewksbury, MA). Two microdialysis fibers (10-mm membrane length, 200-µm diameter, ~35-kDa molecular mass cutoff; MD 2000, Bioanalytical Systems, West Lafayette, IN) were placed in the dermal layer of skin on the ventral surface of the left arm. Fibers were placed with a 25-gauge needle inserted into the skin using sterile techniques. Entry and exit points were ~2.5 cm apart, and the needles were placed ~5 cm apart on the forearm. Microdialysis fibers were threaded through the lumen of the needle, which was then withdrawn, leaving the membrane in place. The fibers were continuously perfused with lactated Ringer solution at a rate of 4 µl/min with a microinfusion pump (Harvard Apparatus Pump, Holliston, MA) during resolution of insertion trauma (90–120 min).

As an index of skin blood flow, cutaneous red blood cell flux was measured directly over the two microdialysis fibers via laser-Doppler flowmetry (Periflux system 5000, Perimed, Stockholm, Sweden). Local skin temperature was controlled with a local heater (Peritemp 4505, Perimed) affixed to the skin over each microdialysis site. Laser-Doppler probes were placed in the center of the local heating units directly above the microdialysis membrane at each skin site. This configuration of local heating units and laser-Doppler probes was used during both protocols and baseline probe temperature was set at 33°C. Probe temperature was maintained at 33°C throughout protocol 2.

To control whole body temperature in protocol 2, subjects wore a tube-lined suit that covered the entire body except the feet and the left forearm. A water-impermeable suit covered the tube-lined suit to limit evaporative heat loss during whole body heating experiments. During microdialysis fiber insertion trauma resolution and baseline conditions, 33°C water was perfused through the suit to maintain thermoneutral conditions. Oral temperature (Toral) was monitored with a sublingual thermistor and mean skin temperature (mean Tsk) was determined by the unweighted average of four copper-constantan thermocouples affixed to the skin on the midthigh, chest, lower back, and abdomen.

**Protocol 1**

A schematic representation of the experimental protocol is depicted in Fig. 1A. The aim of this protocol was to compare the skin blood flow response in subjects with CF and control subjects and to determine the relative contribution of NO-dependent vasodilation during local skin heating. CF subjects (n = 9) and control subjects (n = 9) were instrumented as described above. Site 1 was continuously perfused (4 µl/min) with sterile Ringer solution. Following trauma resolution from fiber insertion, as determined by a consistent plateau in baseline red blood cell flux (90–120 min), site 2 received the NO synthase inhibitor Nω-nitro-L-arginine methyl ester (10 mM, 2.7 mg/ml; L-NAME; Cal Biochem, San Diego, CA) delivered via the microdialysis fiber (4 µl/min) for 30 min before and throughout local heating. The concentration and duration of administration for L-NAME were based on previously published reports establishing the adequacy of the L-NAME dose (4, 11).

Baseline data were collected for 5 min before local heating. The local heating protocol involved increasing skin temperature 0.5°C every 5 s from 33 to 42°C and then maintaining heater temperature at 42°C until skin blood flow reached at the control skin site reached a stable plateau (~45 min; Fig. 1A). This nonpainful skin heating protocol has been shown to elicit consistent and predictable cutaneous

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**Fig. 1.** Schematic diagram of experimental protocols. A: following trauma resolution, site 1 received Ringer solution and site 2 received Nω-nitro-L-arginine methyl ester (L-NAME) for 30 min before and throughout local heating (0.5°C every 5 s to 42°C). B: following trauma resolution, site 1 received Ringer solution and site 2 received L-NAME for 50 min before and throughout whole body heating. △Toral, change in oral temperature.
vasodilation in humans (11). At the end of the local heating protocol, sodium nitroprusside (28 mM, 8.3 mg/ml; Nitropress, Abbot Laboratories, Chicago, IL) was administered (4 μl/ml) for ~45 min to elicit maximal skin blood flow. Although this dose of sodium nitroprusside has been used previously to establish maximal skin blood flow (5, 11, 13) we also increased local skin temperature to 43°C to ensure that maximal cutaneous vasodilation was achieved.

**Protocol 2**

A schematic of the experimental protocol is presented in Fig. 1B. The aim of this protocol was to compare skin blood flow during whole body heating in subjects with CF and controls and to determine the contribution of NO-dependent pathways to cutaneous active vasodilation in subjects with CF. Subjects with CF (n = 9) and control subjects (n = 9) were instrumented as described above. Identical to protocol 1, site 1 was continuously perfused (4 μl/min) with sterile Ringer solution. Following trauma resolution from fiber insertion, as determined by a consistent plateau in red blood cell flux (90–120 min), site 2 received l-NAME (10 mM, 2.7 mg/ml) for 50 min (4 μl/min) before and throughout the whole body heating experiments. The duration of drug infusion was increased for this protocol (relative to protocol 1) to enhance the total l-NAME dose given under baseline conditions (325 μg in protocol 1 vs. 540 μg in protocol 2).

Baseline data were collected for 5 min as thermoneutral water was perfused through the tube-lined suit. Subjects were then heated for 45–60 min, sufficient to raise Tør, 0.8°C (Fig. 1B). Following whole body heating, sodium nitroprusside (28 mM, 8.3 mg/ml) was administered (4 μl/min) for ~45 min at each skin site, as in protocol 1. Local skin temperature was again increased to 43°C to ensure maximal blood flow values were achieved at each skin site.

**Data Acquisition and Analysis**

Data were digitized and stored at 200 Hz on a computer and were analyzed offline using data acquisition software (WinLab, Dataq Instruments, Akron, OH). For analysis, skin blood flow was expressed as cutaneous vascular conductance (CVC) calculated as Doppler flux (mV)/mean arterial pressure (mmHg) and normalized to maximal skin blood flow values (%CVC_max) obtained during sodium nitroprusside administration.

**Protocol 1.** We compared the %CVC_max during the local heating protocol at each skin site in subjects with CF and controls. Values at each phase of the skin blood flow response were determined by averaging laser-Doppler flux over a stable 5-min period at baseline, over a stable 1-min period at the peak, over a stable 1-min period at the nadir, and over a stable 5-min period at the sustained plateau. To compare the contribution of NO-dependent mechanisms during the peak and sustained plateau phase of the skin blood flow response to local heating, we calculated the difference (Δ%CVC_max) between values obtained at site 2 (l-NAME) and values obtained at site 1 (Ringer).

**Protocol 2.** We compared the %CVC_max during the whole body heating protocol at each skin site in subjects with CF and controls. Skin blood flow was determined by averaging laser-Doppler flux values over a stable 5-min period at baseline and over a stable 1-min period at each increase in Tør of 0.2°C. The contribution of the NO-dependent component to the skin blood flow response during whole body heating was calculated the difference (Δ%CVC_max) between values obtained at site 2 (l-NAME) from values obtained at site 1 (Ringer) at each 0.2°C increase in Tør.

**Statistics**

For each protocol, differences within each group (subjects with CF or control subjects) were determined by two-way repeated-measures ANOVA. Differences between groups were determined by independent-measures ANOVA or unpaired t-test where appropriate. Fischer’s least squared difference post hoc tests were used to determine where statistical difference occurred. Values are presented as means ± SE.

**RESULTS**

Subject characteristics for each group are presented in Table 1. Physical characteristics were similar between groups within male or female sex, although male subjects with CF weighed slightly less than their control counterparts (P = 0.1; Table 1).

**Protocol 1.** Baseline CVC was 11 ± %CVC_max in subjects with CF and 9 ± %CVC_max in control subjects (P = 0.2). The administration of NO synthase inhibition did not reduce baseline CVC in subjects with CF (11 ± %CVC_max) or controls (10 ± %CVC_max). Mean (±SE) skin blood flow responses during the local heating protocol are presented in Fig. 2. During local skin heating, a similar skin blood flow pattern existed between control subjects (Fig. 2A) and subjects with CF (Fig. 2B). Local skin heating increased skin blood flow to an initial peak (73 ± 4 vs. 75 ± 3%CVC_max) followed by a nadir (53 ± 6 vs. 58 ± 4%CVC_max) before returning to a sustained plateau (86 ± 3 vs. 81 ± 3%CVC_max) in subjects with CF and controls, respectively (Fig. 2). NO synthase inhibition decreased skin blood flow at each phase of local skin heating to a similar degree in subjects with CF and controls (Fig. 2). The contribution of NO, calculated as difference between control skin sites and sites receiving l-NAME (%CVC_max), during the sustained plateau phase of the skin blood response during local heating was 45 ± 3 and 35 ± 5%CVC_max for CF and control subjects, respectively. Although the contribution of NO to the plateau phase was higher in patients with CF, the difference was not statistically significant (P = 0.1).

**Protocol 2.** Average (±SE) heart rate, mean arterial pressure, Tør, and mean Tsk under normothermic and hyperthermic conditions are presented in Table 2. Hyperthermia increased heart rate (P < 0.01) and mean Tsk (P < 0.01) in both groups, with no statistically different drop in pressure. An unexpected finding was that, under normothermic conditions, Tør was higher in control subjects relative to subjects with CF, which led to a higher absolute Tør obtained by control subjects during hyperthermia (ΔTør of 0.8°C; P = 0.02; Table 2).

To determine the effect of NO synthase inhibition on baseline skin blood flow, we compared baseline CVC values at each site before l-NAME administration in site 2 with CVC

**Table 1. Descriptive characteristics of subjects with CF and control subjects**

<table>
<thead>
<tr>
<th>Subjects with CF</th>
<th>Control Subjects</th>
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<tbody>
<tr>
<td></td>
<td>Men (n = 7)</td>
</tr>
<tr>
<td>Age, yrs</td>
<td>28 ± 3</td>
</tr>
<tr>
<td>Height, cm</td>
<td>179 ± 3</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>74 ± 5</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>23 ± 1</td>
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</table>

Values are means ± SE; n, no. of subjects. CF, cystic fibrosis.
values at each site following 50 min of L-NAME administration or 50 min of Ringer infusion in site 1. There was no statistical difference between skin sites (Ringer: 16 ± 2% CVCmax vs. L-NAME: 12 ± 1% CVCmax; P = 0.1) of subjects with CF before L-NAME infusion in site 2. Similarly, there was no statistical difference between sites in control subjects before L-NAME infusion in site 2 (Ringer: 10 ± 2% CVCmax vs. L-NAME: 9 ± 1% CVCmax; P = 0.6). In subjects with CF, 50 min of Ringer infusion (site 1) did not reduce CVC (16 ± 2 vs. 16 ± 2% CVCmax; P = 0.6), and 50 min of L-NAME infusion did not reduce CVC (12 ± 1 vs. 11 ± 2% CVCmax; P = 0.2). In control subjects, 50 min of Ringer infusion (site 1) did not reduce CVC (10 ± 2 vs. 10 ± 1% CVCmax; P = 0.4), and 50 min of L-NAME infusion (site 2) did not reduce CVC (9 ± 1 vs. 8 ± 1% CVCmax; P = 0.2).

Presented in Fig. 3 are the group data demonstrating the increase in CVC with increasing core body temperature in skin sites receiving Ringer solution and sites receiving L-NAME. A rise in Tor of 0.8°C increased CVC at skin sites receiving Ringer solution to 53 ± 4% CVCmax in control subjects and to 58 ± 4% CVCmax in subjects with CF (Fig. 3; P = 0.4). In skin sites receiving L-NAME, a rise in Tor of 0.8°C increased CVC to 35 ± 5% CVCmax in control subjects and 37 ± 6% CVCmax in subjects with CF (Fig. 3; P = 0.8). The contribution of NO to cutaneous active vasodilation (Δ% CVCmax) with an elevation in Tor of 0.8°C in was similar between subjects with CF (22 ± 7% CVCmax) and control subjects (18 ± 4% CVCmax; P = 0.8).

DISCUSSION

The primary findings from this study were that subjects with CF have a normal skin blood flow pattern to local skin heating.
with a robust NO-dependent vasodilation (Fig. 2). In addition, cutaneous active vasodilation during whole body heating (rise in \( T_{or} 0.8\)°) was similar in subjects with CF and control subjects (Fig. 3). Contrary to our hypothesis, the NO-dependent portion of active vasodilation was similar between subjects with CF and controls. The preserved active vasodilation and similar contribution of NO-dependent pathways occurred despite the potential scarcity of VIP-immunoreactive nerve fibers in the skin of subjects with CF (16). Combined, the results from the experiments reported here extend the previous study that examined cutaneous vascular control during local and whole body heating in subjects with CF. That is, our results quantified the contribution for NO-dependent mechanisms and suggest that enhanced NO-dependent mechanisms do not account for the preserved cutaneous active vasodilation in subjects with CF.

Vasodilation to Local Skin Heating

Our reasoning for investigating the skin blood flow responses during local heat application was to examine a potential upregulation of NO-dependent mechanisms in the skin of subjects with CF. This hypothesis was based on results from Savage et al. (16) that suggest a greater blood flow response to localized forearm heating in subjects with CF. The mechanisms for heat-evoked vasodilation in human skin are unknown. Heat-sensitive nociceptors are activated in response to heat stimuli in human skin (9) and the neuropeptides calcitonin gene-related peptide and substance P are thought to be released from these nociceptive afferents in animal models (8, 15). If this is true in human skin, it is not surprising that subjects with CF had a similar skin blood flow pattern to control subjects, as these two peptides are abundant in cutaneous nerve of subjects with CF (16). Our results demonstrate that subjects with CF have a robust NO-dependent vasodilation during the sustained plateau phase of the local heating protocol, but the contribution of NO was not statistically higher than control subjects (\( P = 0.1 \)).

Cutaneous Active Vasodilation

VIP was generally dismissed as the substance responsible for cutaneous active vasodilation in human skin by Savage et al. (16), when they demonstrated similar cutaneous active vasodilation between subjects with CF and controls. More recently, interest for VIP as the vasodilator signal for cutaneous active vasodilation came from a report suggesting the VIP receptor antagonist VIP10–28 inhibited a portion of active vasodilation (1). However, the efficacy of the VIP10–28 antagonist during active vasodilation in human skin has been questioned (20).

Savage et al. (16) acknowledged that the preserved cutaneous active vasodilation observed in subjects with CF must be possible notwithstanding sparse VIP immunoreactivity. In this context, recent data suggest that the NO-dependent and NO-independent components of cutaneous active vasodilation are not simply additive. That is, NO may interact synergistically with another vasodilator and their combined affect will ultimately dictate cutaneous blood flow during whole body heating (19). Furthermore, their vasodilation to exogenous VIP includes a substantial NO-dependent portion (18). It is therefore possible that sparse VIP content in cutaneous nerves is offset by an up-regulation in NO-dependent vasodilation in subjects with CF. However, results from the present study demonstrate a similar NO-dependent portion of active vasodilation in subjects with CF and controls (Fig. 3). This finding, combined with previous reports of deficient VIP-content in cutaneous nerves of patients with CF (3, 16), suggests the preservation of cutaneous active vasodilation in patients with CF is not due to an enhanced NO-dependent mechanism potentially compensating for the loss of a direct VIP-mediated component.

We were unable to determine VIP-immunoreactivity in the skin of our subject groups and were unable to quantify VIP release in human skin. To our knowledge, the later has never been accomplished in human skin and would be the only direct indicator of a role for VIP. It is possible that the cutaneous nerves in the skin of our group of CF subjects were not VIP-deficient. However, this seems unlikely due to the frequency of the \( \Delta F508 \) mutation, as the dominant genetic mutation of CF (14). Additionally, sparse VIP-immunoreactivity in cutaneous nerves may not translate into reduced VIP release during whole body heating. Without quantification of VIP release, studies examining potential VIP-related mechanisms for cutaneous active vasodilation in human skin will not be entirely conclusive.

Several vasodilator substances contribute to cutaneous active vasodilation in human skin. In addition to NO, these substances include, but may not be limited to, prostaglandins (10), histamine (\( H_1 \) receptors; 22) and substance P (21). Although the relative contribution of the multiple vasodilator substances have been examined (4, 10, 22), the potential interactions and possible redundancies for the multiple vasodilator pathways are unknown. For instance, inhibition of a single vasodilator pathway may be offset by the activation or upregulation of another. This has been examined in skeletal muscle (17) but, to our knowledge, has not been studied comprehensively in human skin. An enhanced contribution from prostaglandins or \( H_1 \) receptor activation may account for the preserved reflex cutaneous vasodilation in subjects with CF. The neuropeptide substance P, shown to be profuse in cutaneous nerves of patients with CF has been recently identified as a potential vasodilator signal mediating active vasodilation in human skin (21). NO synthase inhibition attenuates substance P-mediated vasodilation in human skin (7), suggesting a potential role for substance-P mediated NO release during active vasodilation. It is possible that substance P-mediated vasodilation; normally plays a substantial role in skin blood flow responses to reflex vasodilation or is upregulated in subjects with CF. An important consideration here is that substance-P has not been identified in sympathetic active vasodilator nerves in human skin and is usually associated with sensory nerve endings (8). The potential link between sensory nerve afferents and sympathetic active vasodilator nerves remains to be identified.

Limitations

As discussed above, we were unable to establish deficient VIP content in cutaneous nerves or quantify VIP release from the skin of subjects with CF participating in our study. However, the \( \Delta F508 \) mutation accounts for 70% of the chromosomes of individuals with CF, where other mutations are very
H1 receptor activation contributes to active vasodilation (22), the preserved active vasodilation of CF subjects suggest the H1 receptor antagonist did not influence our findings. To our knowledge, there is no available information related to the effect of antibiotics on cutaneous vascular control in humans.

In conclusion, findings from the present study suggest the NO-dependent portion of thermal cutaneous vasodilation in subjects with CF is robust. However, the preserved cutaneous active vasodilation of subjects with CF is not due to an enhanced NO-dependent component, despite reports of deficient VIP immunoreactivity in cutaneous nerves. Thus our findings suggest the preserved cutaneous active vasodilation in subjects with CF may be due to the activation of a redundant vasodilator mechanism in the absence of NO-dependent pathways or the upregulation another NO-independent vasodilator substance such as prostaglandins, histamine, or substance P.

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