Mechanisms of vasoactive intestinal peptide-mediated vasodilation in human skin

Brad W. Wilkins, Linda H. Chung, Nathan J. Tublitz, Brett J. Wong, and Christopher T. Minson. Mechanisms of vasoactive intestinal peptide-mediated vasodilation in human skin. J Appl Physiol 97: 1291–1298, 2004. First published May 21, 2004; 10.1152/japplphysiol.00366.2004.—Vasoactive intestinal peptide (VIP) is known to induce histamine release in human skin and to include a nitric oxide (NO)-dependent dilation in several other vascular beds. However, the relative contribution of histamine and NO to VIP-mediated vasodilation in human skin is unknown. Forty-three subjects volunteered to participate in two studies designed to examine the mechanism of VIP-mediated vasodilation in human skin. Study 1 examined the contribution of NO in the skin blood flow response to eight doses of VIP ranging from 25 to 800 pmol. In addition, study 1 examined a specific role for NO in VIP-mediated dilation. Study 2 examined the relative contribution of NO and histamine to VIP-mediated dilation via H1 and H2 histamine receptors. Infusions were administered to skin sites via intradermal microdialysis. The use of microdialysis as the method for peptide delivery would curtail any potentially confounding effects of intradermal injection on histamine release from skin mast cells.

The action of histamine in human skin has been described, where intradermal histamine injection elicits a wheal and flare reaction (6, 8, 13). Antagonists of the H1 isoform of the histamine receptor attenuate this wheal and flare reaction to histamine injection (13, 16, 40, 45). Cutaneous vasodilation via the H2 isoform of the histamine receptor has also been described, demonstrating an attenuated vasodilation to the iontophoretic administration of histamine (20, 21) and intradermal histamine injection (15, 16, 27). Interestingly, intradermal histamine injection generates measurable (via microdialysis) increases in nitric oxide (NO) production in human skin (6, 7), contributing to a portion of the histamine-mediated vasodilation (6). In the context of VIP-mediated vasodilation, it is possible that histamine-stimulated NO production is an important contributor to VIP-mediated vasodilation. Along these lines, VIP-mediated vasodilation includes important NO-dependent pathways in the human uterine artery (28). To our knowledge, no study has specifically examined the participation of NO and/or the relative contributions of histamine and NO to VIP-mediated vasodilation in human skin.

In gastric smooth muscle, it is well established that VIP-induced relaxation is due, in part, to NO-dependent mechanisms (17, 26). The NO portion of VIP-mediated gastric smooth relaxation has been shown to include an interaction between VIP and NO at some point in the signaling pathway (9, 17, 18, 26). In a guinea pig model, responses in airway smooth muscle were due to a “synergistic” interaction between NO and VIP. That is, the combined action of NO and VIP elicits a response that is greater than the sum of the individual responses (29). In the context of human skin, our laboratory has demonstrated a synergistic role for NO in thermoregulatory active vasodilation (49). If VIP-mediated vasodilation in hu-
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man skin includes a NO-dependent component, it is possible that a synergistic interaction is present between these two substances in human skin. Because VIP is an important neurotransmitter in cutaneous nerves, it is necessary to understand the mechanism of VIP-mediated dilation.

With the use of this information as background, the purpose of this study was to systematically examine the mechanism of VIP-mediated vasodilation in human skin to identify the relative contributions of NO and histamine in VIP-mediated dilation. We performed two studies intended to determine the mechanism of vasodilation in the cutaneous vasculature to exogenous VIP. Specifically, the studies were designed to test the following hypotheses. First, cutaneous vasodilation to exogenous VIP administered via microdialysis includes the activation of NO-dependent pathways, where NO acts in a synergistic manner to augment VIP-mediated vasodilation. Second, vasodilation to exogenous VIP includes the activation of H1 and H2 receptors in human skin, where histamine binding accounts for VIP-induced NO production and the NO-dependent portion of VIP-mediated vasodilation.

METHODS

Subjects. Nineteen women (mean age 23 ± 1 yr) and 24 men (mean age 22 ± 1 yr) volunteered to take part in two separate studies. Institutional Review Board approval was obtained, and each subject gave informed consent before participation. All subjects underwent standard health screening and were healthy, normotensive, nonsmokers. Institutional Review Board approval was obtained, and each subject gave informed consent before participation. All subjects underwent a standard health screening and were healthy, normotensive, nonsmokers, and not taking any medications, with the exception of oral contraceptives.

Instrumentation. Subjects were instrumented with an electrocardiogram, which was monitored throughout the protocol with a CardioCap monitor (Datex-Ohmeda, Tewksbury, MA), and arterial pressure was assessed by brachial auscultation every 5 min. All protocols were performed in a temperature-controlled laboratory maintained at 23.5 ± 0.5°C.

Microdialysis fibers (model MD 2000, Bioanalytical Systems, West Lafayette, IN) with a membrane length of 10 mm and molecular mass cutoff of 20 kDa (<5-μl dead space) were placed in the skin of the ventral aspect of the nondominant forearm. Microdialysis probes were placed with a 25-gauge needle inserted through the dermis of the skin using sterile techniques in the absence of anesthesia. The probe was then threaded through the internal lumen of the needle and the needle was withdrawn, leaving the membrane in place. The fiber was taped in place and perfused with lactated Ringer solution at a rate of 2 μl/min with a microinfusion pump (Harvard Apparatus, Holliston MA; and CMA/102, CMA Microdialysis, Stockholm, Sweden). Sites were at least 5 cm apart.

To obtain an index of skin blood flow, cutaneous red blood cell flux was measured directly over each microdialysis site by laser-Doppler flowmetry (LDF; MoorLAB, Moor Instruments, UK) with integrated laser-Doppler probes fixed to the skin with adhesive tape. The integrated probes have eight collecting fibers placed in a 2-mm ring around a single emitting fiber. After placement of the microdialysis fibers, skin blood flow was monitored to determine that the insertion trauma had resolved before each protocol began (between 90 and 150 min).

Experiments. Figure 1 (A and B) displays the general experimental design for studies 1 and 2. Study 1 was designed to determine the participation of NO and to examine a specific role for NO in VIP-mediated vasodilation in human skin. Initially, a group of 12 subjects were instrumented with four microdialysis fibers, where each site was assigned to receive a single dose of VIP (CalBiochem, San Diego, CA) dissolved in lactated Ringer solution. Collectively, each dose of VIP was infused through a skin site in six subjects. Baseline Ringer infusion was followed by administering a single dose of VIP at 25, 50, 100, 200, 300, 400, 500, or 800 pmol delivered through each microdialysis fiber in a total volume of 2 μl over 30 s. That is, the infusion rate was increased to 4 μl/min to deliver a bolus picomole dose of VIP. From pilot experiments, we determined that a continuous infusion of VIP (μmol/l) did not produce a consistent and repeatable plateau in skin blood flow. Skin blood flow rose throughout the continuous infusion, never reaching a stable plateau value, independent of VIP concentration in the perfusate. Furthermore, the rate of rise in skin blood flow was not dependent on concentration. For example, a continuous infusion of VIP at a concentration of 25 μM for 1 h (12 μl/min) increased skin blood flow from 10 to 67% of maximal cutaneous vascular conductance (CVCmax%). When the infusion was stopped at 60 min, skin blood flow rose to 83%CVCmax% after monitoring for another 30 min (90 min after infusion began). In the same subject, a continuous infusion of VIP at a concentration of 50 μM for 1 h (2 μl/min) increased skin blood flow from 10 to 62%CVCmax%, which, similar to the 25 μM infusion, continued to rise to 85%CVCmax% 30 min after infusion stopped. Ten pilot subjects (not considered part of the 43 subjects in this report) were studied while the rate and duration of VIP dose delivery were varied to enable the determination of a stable plateau in skin blood flow within a reasonable time period. A single (30 s) picomole dose of VIP produced a stable plateau in skin blood flow 1.5–2.0 h after infusion. Therefore, to investigate the contribution of NO to VIP-mediated dilation, we administered a brief (pmol) dose of exogenous VIP to each skin site. After administration of VIP, infusion of Ringer solution continued throughout the remainder of the protocol (Fig. 1A). Red blood cell flux was measured throughout the baseline period and for 1.5–2 h after administration of the VIP dose.

To examine the contribution of NO to VIP-mediated dilation, an additional group of 12 subjects were instrumented with four microdialysis fibers. Each microdialysis site received a 30-min infusion (10 mM at 2.0 μl/min) of the NO synthase inhibitor Nω-nitro-L-arginine methyl ester (l-NAME; CalBiochem) dissolved in Ringer solution. The continuous infusion of this dose of l-NAME has been shown to sufficiently block endothelial NO production, where increasing the concentration of l-NAME does not further reduce the skin blood flow response known to be mediated by NO (31, 38). Eight equivalent doses of VIP to those used in the protocol above were administered, with each skin site receiving a single dose of VIP. Infusion of l-NAME continued for the remainder of the protocol after the administration of the VIP dose (Fig. 1A). Red blood cell flux was measured throughout the baseline period and for 1.5–2 h after VIP infusion.

To examine the specific role for NO in VIP-mediated dilation, a group of six subjects were instrumented with two microdialysis fibers randomly assigned to receive a constant infusion of either a low dose (0.5 μM) of sodium nitroprusside (SNP; Nitropress, Abbott Laboratories, Chicago, IL) or a low dose of SNP with l-NAME (final concentrations of 0.5 μM SNP and 10 mM l-NAME). This dose of SNP was chosen because our laboratory has previously determined that it is sufficient to produce minimal dilation under baseline conditions, and it was used previously in our laboratory to study the role of NO during whole body heating in humans (49). Baseline Ringer infusion was followed by continuous low-dose SNP infusion (site 1) or continuous infusion of low-dose SNP with l-NAME (site 2) for 30 min (2 μl/min). At this point, a single 200-pmol dose of VIP was administered to each skin site. After delivery of VIP, the low-dose SNP or the low-dose SNP with l-NAME infusions continued throughout the remainder of the protocol (Fig. 1A). The 200-pmol dose of VIP was used for this protocol because this dose produces a substantial skin blood flow response that was below the saturable doses as determined in the initial protocol, and the vasodilation produced from this dose of VIP had a significant NO-dependent component. Red blood cell flux was measured during baseline, low-dose SNP infusion, and for 1.5–2 h after VIP administration.
The goal of this protocol was to examine whether NO interacts with VIP in a synergistic manner. That is, if continuous low-dose SNP augments VIP-mediated vasodilation in a site in which NO synthase was inhibited (which cannot be explained by the low-dose SNP alone), we would conclude that NO interacts in a synergistic manner. Alternatively, if continuous low-dose SNP infusion does not augment VIP-mediated dilation, we would conclude that there is no synergistic interaction between NO and exogenous VIP.

Study 2 was designed to determine the relative contributions of NO and histamine (through H1- and H2-receptor activation) in VIP-mediated vasodilation. We performed pilot protocols to ensure the H1-receptor antagonist pyrilamine maleate (500 μM; Sigma Chemical, St. Louis, MO) and the H2-receptor antagonist cimetidine (2 mM; Sigma Chemical) competitively antagonized H1- and H2-receptor-mediated vasodilation. A 5-pmol dose (volume of 2 μl over 30 s) of histamine (Sigma Chemical) was administered through microdialysis fibers after 30 min of pyrilamine infusion (4 subjects) and 30 min of cimetidine infusion (4 subjects). Figure 2 displays data from these pilot studies, demonstrating effective competitive inhibition of H1- and H2-receptor-mediated vasodilation. Pyrilamine maleate has been shown previously to significantly inhibit histamine release from intradermal injections of VIP (13), and cimetidine has been shown previously to inhibit histamine-mediated vasodilation (20) and the wheal and flare reaction (27).

To examine the potential contribution of H1 receptors in the NO portion of VIP-mediated vasodilation, a group of six subjects were instrumented with three microdialysis fibers. Skin sites were randomly assigned as a control site (Ringer only), a site receiving a 30 min infusion (500 μM and 10 mM, respectively) of pyrilamine combined with L-NAME (final concentrations of 500 μM and 10 mM, respectively) dissolved in Ringer solution (Fig. 1B). Baseline drug infusions were followed by a single 200-pmol dose of VIP at each microdialysis site (as in study 1). Respective drug infusion at each site.
continued after administration of the VIP dose for the remainder of the protocol (Fig. 1B). Red blood cell flux was measured throughout the baseline period and for 1.5–2 h after VIP infusion.

To examine the participation of H2 receptors, the relative contribution of H1 and H2 receptors, and the potential contribution of H2 receptors in the NO portion of VIP-mediated vasodilation, an additional group of six subjects were instrumented with three microdialysis fibers. Skin sites were randomly assigned as a control site (Ringer only), a site receiving a 30-min infusion (2 mM at 2 μl/min) of cimetidine dissolved in Ringer solution, a site receiving a 30-min infusion of cimetidine combined with pyrilamine (final concentrations of 2 mM and 500 μM, respectively), and a site receiving a 30-min infusion of cimetidine combined with l-NAME (final concentrations of 2 mM and 10 mM respectively) dissolved in Ringer solution (Fig. 1B). Baseline drug infusions were followed by a single 200-pmol dose of VIP at each microdialysis site, and drug infusions continued for the remainder of the protocol after administration of the VIP dose (Fig. 1B). Red blood cell flux was measured throughout the baseline period and for 1.5–2 h after VIP infusion.

At the end of all experiments, 28 mM SNP was infused (4 μl/min) to maximally vasodilate the skin at each microdialysis site to enable comparison of relative skin blood flow values between protocols. This dose has previously been shown to elicit maximal skin blood flow (31, 38).

Data acquisition and analysis. Data were digitized and stored at 20 Hz on a computer and were analyzed offline by using signal processing software (Windaq, Dataq Instruments, Akron, OH). Skin blood flow was assessed by averaging LDF values over stable 5-min periods. For data analysis, skin blood flow was expressed as cutaneous vascular conductance (CVC), calculated as LDF (mV)/mean arterial pressure (MAP; mmHg), and normalized to the maximal levels achieved during infusion of 28 mM SNP.

To assess the response to exogenous VIP in study 1, CVC values were determined in all experiments at the established plateau in skin blood flow after the administration of each VIP dose. To compare the skin blood flow response to exogenous VIP infusion and to examine the contribution of NO to VIP-mediated dilation at each dose, plateau CVC values were compared by a two-way ANOVA. To examine the specific role of NO in VIP-mediated dilation, the response to a 200-pmol dose of VIP in skin sites receiving continuous low-dose SNP with l-NAME were compared with skin sites receiving a 200-pmol dose of VIP with l-NAME alone by Student’s t-test. Furthermore, the response to a 200-pmol dose of VIP in skin sites receiving the low-dose SNP infusion were compared with Student’s t-test to those sites that only received a 200-pmol dose of VIP.

To assess the response to exogenous VIP infusion in study 2, CVC values were obtained at the established plateau in skin blood flow from a 200-pmol dose of VIP at each site. To establish the relative contribution for NO and H1-receptor activation to VIP-mediated vasodilation, the response to a 200-pmol dose of VIP in control skin sites were compared with sites receiving H2 antagonist alone and H1 antagonist together with NO synthase inhibitor. In addition, the analysis included the response from sites receiving a 200-pmol VIP dose with NO synthase inhibition during study 1. The CVC values from VIP administration during these drug manipulations were compared by one-way independent-measures ANOVA. To establish the contribution of H1- and H2-receptor activation to VIP-mediated vasodilation, the response to a 200-pmol dose of VIP in control sites was compared with sites receiving H2 antagonist alone, sites receiving combined H1 and H2 antagonist, and sites receiving H2 antagonist together with l-NAME. The CVC values from VIP administration during these drug manipulations were compared by one-way repeated-measures ANOVA.

For all statistical tests, a Fisher’s least significant difference post hoc test was used to determine where differences occurred, and associated P values of <0.05 were considered statistically significant. All values are presented as means ± SE.

RESULTS

Study 1. Presented in Fig. 3A are representative tracings of two skin sites (50- and 200-pmol doses of VIP) from one subject. These representative tracings demonstrate the common response and plateau in CVC observed for all eight doses of VIP. Presented in Fig. 4 are the group data (each point displays the plateau value for each VIP concentration) from the eight doses of exogenous VIP alone and from the eight doses of VIP with NO synthase inhibition. The infusion of exogenous VIP via microdialysis fibers produced a dose-dependent vasodilation in human skin, where incremental doses of VIP significantly increased CVC (P < 0.01). Importantly, the dose-dependent nature of the skin blood flow response to exogenous VIP infusion was saturable, where doubling the VIP dose (400 vs. 800 pmol) did not significantly increase CVC (P = 0.43).

Presented in Fig. 3B are representative tracings of two skin sites (doses of 50 and 200 pmol) from one subject in which NO synthase was inhibited. Importantly, there was a NO-dependent component to VIP-mediated dilation at doses of VIP above 50 pmol. That is, VIP-mediated dilation was attenuated during NO synthase inhibition at VIP doses of 100 pmol or greater (Fig. 4; P < 0.01). The NO-independent portion of VIP-mediated dilation was also dose dependent, because there was a main effect on CVC with incremental doses of VIP during NO synthase inhibition (P = 0.04). Importantly, the NO-independent vasodilation produced by exogenous VIP at the 200-pmol dose and above were saturated, as there was no difference between these doses (Fig. 4; P = 0.23).

Figure 5 displays the group data from the administration of a 200-pmol dose of VIP during continuous low-dose SNP and group data from the administration of the 200-pmol dose of VIP during continuous low-dose SNP with l-NAME. These responses are compared with the group data from the 200-pmol VIP dose alone and the group data from the 200-pmol VIP dose delivered during NO synthase inhibition. A 200-pmol VIP dose administered during continuous low-dose SNP increased CVC...
to 63 ± 7%\text{CVC}_{\text{max}}, \text{ which was similar to the infusion of this dose of VIP alone (64 ± 4%\text{CVC}_{\text{max}}; P = 0.83). Similarly, a 200-pmol VIP dose during continuous low-dose SNP with L-NAME infusion increased CVC to 38 ± 5%\text{CVC}_{\text{max}}, \text{ which was similar to the 200-pmol VIP dose infused during NO synthase inhibition (35 ± 8%\text{CVC}_{\text{max}}; Fig. 5; P = 0.78). These data suggest that there is no postjunctional synergistic interaction between NO and VIP at any point along the signaling pathway. }

\textbf{Study 2.} The infusion of the H}1\text{ antagonist (500 } \mu \text{M pyrilamine) alone and H}1\text{-receptor antagonist with NO synthase inhibition for 30 min had no effect on baseline skin blood flow. The infusion of H}1\text{-receptor antagonist inhibited the rise in CVC to a 200-pmol VIP dose (32 ± 10 vs. 65 ± 6%\text{CVC}_{\text{max}} \text{ in control sites; } P < 0.01). The blunted rise in CVC with H}1\text{-receptor inhibition was similar to the CVC response to a 200-pmol VIP dose with NO synthase inhibition (35 ± 8%\text{CVC}_{\text{max}}; P = 0.5). It appears that the NO-dependent portion of VIP-mediated vasodilation cannot be entirely explained by H}1\text{-receptor activation. That is, CVC to 200-pmol VIP in sites receiving the combined H}1\text{-receptor antagonist and NO synthase inhibition (13 ± 2%\text{CVC}_{\text{max}}) was lower than sites receiving either H}1\text{-receptor antagonist alone or NO synthase inhibition alone (}P = 0.04\text{ and } P < 0.01, \text{ respectively).}

The infusion of H}2\text{ antagonist alone, H}2\text{-antagonist together with NO synthase inhibition, and combined H}2\text{ and H}1\text{ antagonists for 30 min had no effect on baseline skin blood flow. Interestingly, infusion of H}2\text{ antagonist alone had no effect on CVC to a 200-pmol dose of VIP (53 ± 7 vs. 55 ± 3%\text{CVC}_{\text{max}} \text{ in control sites; } P = 0.99). Infusion of H}2\text{-receptor antagonist together with NO synthase inhibition attenuated the rise in CVC to a 200-pmol VIP dose (37 ± 5%\text{CVC}_{\text{max}}) compared with control sites (}P = 0.03). Similar to the infusion of H}1\text{-receptor antagonist with NO synthase inhibition.}

VIP in sites receiving the combined H}1\text{-receptor antagonist and NO synthase inhibition (13 ± 2%\text{CVC}_{\text{max}}) was lower than sites receiving either H}1\text{-receptor antagonist alone or NO synthase inhibition alone (}P = 0.04\text{ and } P < 0.01, \text{ respectively).}

The infusion of H}2\text{ antagonist alone, H}2\text{-antagonist together with NO synthase inhibition, and combined H}2\text{ and H}1\text{ antagonists for 30 min had no effect on baseline skin blood flow. Interestingly, infusion of H}2\text{ antagonist alone had no effect on CVC to a 200-pmol dose of VIP (53 ± 7 vs. 55 ± 3%\text{CVC}_{\text{max}} \text{ in control sites; } P = 0.99). Infusion of H}2\text{-receptor antagonist together with NO synthase inhibition attenuated the rise in CVC to a 200-pmol VIP dose (37 ± 5%\text{CVC}_{\text{max}}) compared with control sites (}P = 0.03). Similar to the infusion of H}1\text{-receptor antagonist with NO synthase inhibition. *}P < 0.05\text{ vs. VIP alone.}

\textbf{Fig. 4.} Mean (± SE) CVC responses to 25-, 50-, 100-, 200-, 300-, 400-, 500-, and 800-pmol doses of VIP alone and the same eight doses of VIP during NO synthase inhibition. Responses were measured at the established plateau in skin blood flow after the delivery of each VIP dose. Incremental doses of VIP produced a dose-dependent dilation in the cutaneous vasculature. In addition to a NO-dependent component, VIP-mediated vasodilation includes a NO-independent portion that was dose dependent. *}P < 0.05\text{ vs. VIP alone.}

\textbf{Fig. 5.} Mean (± SE) CVC responses in sites receiving a 200-pmol dose of VIP during low-dose SNP experiments. Responses were measured at baseline, during continuous low-dose SNP infusion, at the plateau in skin blood flow after a 200-pmol dose of VIP alone, and at the plateau in skin blood flow after a 200-pmol dose of VIP during continuous low-dose SNP infusion. Exogenous NO did not augment VIP-mediated vasodilation during NO synthase inhibition. *}P < 0.05\text{ vs. baseline CVC.}
antagonist alone, combined H₁- and H₂-receptor antagonists attenuated CVC to a 200-pmol dose of VIP (30 ± 6% CVCmax) compared with control sites (P = 0.02). Sites receiving combined H₂- and H₁-receptor antagonist were similar to skin sites receiving combined H₂ antagonist with NO synthase inhibition (P = 0.99).

**DISCUSSION**

The studies presented here are the first to examine the relative contribution of NO and H₁ and H₂ histamine receptors in VIP-mediated vasodilation. We found that, in addition to a NO-independent component, VIP-mediated dilation in human skin includes a substantial NO portion at doses of VIP at and above 100 pmol (Fig. 4). Contrary to our hypothesis in study 1, regarding a potential synergistic role of NO in VIP-mediated dilation, the NO-dependent component of VIP-mediated vasodilation does not appear to be due to a synergistic interaction between exogenous VIP and NO (Fig. 5). Consistent with reports using intradermal injection, our results from study 2 suggest that vasodilation to exogenous VIP, administered via microdialysis, includes an H₁-receptor-mediated component. Our results from study 2 extend these findings to suggest that the H₂ isoform of the histamine receptor does not contribute to VIP-mediated vasodilation in human skin. Furthermore, H₁-receptor activation could not explain the entire NO-dependent portion of VIP-mediated vasodilation. This suggests that, in addition to a potential histamine-induced NO portion, a histamine-independent NO component contributes to VIP-mediated vasodilation.

**VIP in human skin.** Immunoreactive nerve fibers for VIP (44, 47) and VIP receptors (12, 35) are present in human skin, concentrated around arterioles and sweat glands. Several studies have examined the participation of VIP in cutaneous vasodilator responses, mostly the inflammatory wheal and flare reaction to intradermal VIP injection (1, 3, 13, 42, 44). The wheal and flare reaction to intradermal VIP injection in human skin is known to be mediated, in part, through mast cell degranulation and the release of histamine (13, 23, 37, 39, 41). Moreover, VIP receptors have recently been identified on mast cells in human skin (19). The prevalence of this neuropeptide and its receptor around arterioles and sweat glands suggests a potentially important role for VIP in human skin that is independent of the inflammatory skin response. Recent evidence suggests a role for VIP in thermoregulatory active vasodilation in human skin (2). In this context, it is well established that thermoregulatory active vasodilation includes an important NO-dependent component (30, 32, 48, 49). In support of a role for VIP in active vasodilation, the present study clearly establishes an important NO-dependent component in VIP-mediated vasodilation. Information regarding the mechanisms of VIP-mediated vasodilation in human skin is lacking. To our knowledge, this study is the first to demonstrate a dose-dependent and saturable increase in skin blood flow to an exogenous source of VIP administered via microdialysis in human skin (Fig. 4).

**NO and histamine-receptor components of VIP-mediated vasodilation.** A number of studies have identified the contribution of NO in VIP-mediated relaxation of gastric smooth muscle (9, 17, 26) and in VIP-mediated vasodilation (14, 22, 24, 28). Similarly, the results from this study demonstrate that in human skin there is a substantial NO-dependent component to VIP-mediated vasodilation. NO synthase inhibition attenuated, but did not abolish, the skin blood flow response to exogenous VIP doses at or above 100 pmol (Fig. 4). These results are similar to those obtained from other basic peptides, namely substance P and calcitonin gene-related peptide (CGRP), which induce NO release from microvascular endothelial cells from human skin (4, 33).

A potential source for NO in VIP-mediated dilation is through histamine secretion from skin mast cell degranulation. In this context, histamine-mediated vasodilation to intradermal histamine injection has been shown to be due, in part, to the production of NO (6, 7). The results from this study suggest that histamine induced NO production could not account for the entire NO-dependent portion of VIP-mediated vasodilation in human skin. In fact, whereas vasodilation to a 200-pmol dose of VIP was reduced during H₁-receptor antagonist infusion alone to a level similar to NO synthase inhibition alone, the action of these combined antagonists further reduced VIP-mediated dilation. This suggests that vasodilation to exogenous VIP includes a NO-dependent component that is due to a histamine-independent VIP-induced NO production. This conclusion is based on the assumption that this dose of H₁-receptor antagonist inhibited histamine binding at the concentrations of histamine produced by exogenous VIP infusion. Because of the substantial difference between the skin blood flow response with combined H₁ antagonist and NO synthase inhibition compared with either antagonist alone, a clear histamine-independent source for NO is evident. In addition to the NO-dependent portion of VIP-mediated vasodilation, our data suggest that activation of H₁ receptors is responsible for a portion of VIP-mediated vasodilation. However, it remains unclear whether there was any direct vasodilation from VIP, independent of the NO and H₁ components. Interestingly, combined H₁ antagonist and NO synthase inhibition explain the majority of the vasodilation to exogenous VIP.

In addition to the direct effect of VIP and NO on smooth muscle through their individual pathways, relaxation in gastric smooth muscle may be due to a convergent interaction between their respective second-messenger pathways, cAMP for VIP and cGMP for NO (17, 26). In vascular smooth muscle, second messenger “cross talk” between the cAMP and cGMP pathways (5, 11) may play an important physiological role in vasodilation. In the present study, although the response to a 200-pmol dose of VIP includes a substantial NO component (Fig. 4), the addition of exogenous NO failed to augment the dilation above the NO-independent component (Fig. 5). Therefore, contrary to our original hypothesis, our results suggest NO does not interact in a synergistic manner with VIP in human skin. However, it is possible that endogenous NO may enhance the release of VIP from nerve endings (18). Because we only examined the vasodilation to exogenous VIP in the present study, it is unknown whether NO prejunctionally enhances endogenous VIP release in human skin.

In human skin, vasodilation via H₂-receptor activation has been demonstrated during the iontophoretic administration of histamine (20), as well as intradermal histamine injection (15, 16, 27). We demonstrated substantial antagonist affects on H₂ receptor activation during histamine administration via microdialysis (Fig. 2). Interestingly, our data suggest that H₂-receptor activation does not contribute to VIP-mediated dilation in
human skin. Of interest here is the potential difference in the mechanism of action of H₁ and H₂ receptors. That is, in human extracranial arteries H₁-receptor activation evokes NO release from vascular endothelium (25), and human H₁ receptors increase Ca²⁺ concentration (10). In contrast, H₂ receptor activation of human internal mammary arteries leads to direct smooth muscle relaxation and vasodilation (46), probably through cAMP production via adenylate cyclase activation (10). Our data suggest a portion of the NO-dependent component of VIP-mediated dilation includes the H₁-receptor activation of NO production.

**Limitations.** A potential limitation to microdialysis studies in human skin is quantifying the concentration of drug applied to the skin at the dialysis membrane. In our laboratory, we have been unable to assess VIP recovery in vivo using immunochemical techniques. However, incremental doses of VIP delivered through the microdialysis fiber produced a consistent, dose-dependent, and repeatable vasodilation in human skin. Therefore, we are confident that we delivered eight doses of VIP at the dialysis membrane, which were proportional to the concentration of the dose in the perfusate (25–800 pmol).

The time course of VIP-mediated vasodilation in human skin has not been well defined. Huttunen et al. (23) reported wheal and flare reactions to intradermal VIP injection occurring 5–10 min after injection, and skin reactions to intradermal VIP injection have been reported to persist for 2–3 h after injection (3). In contrast, peak flare reactions have been reported at ~3 min after intradermal injection, whereas the peak wheal reaction was reported to be ~9 min (41). In the present study, the onset of dilation after dose delivery was delayed somewhat (15 ± 2 min), and the time to the plateau vasodilation was 75 ± 2 min after dose administration. Administration of peptides via microdialysis requires diffusion into the intradermal space, which would lengthen the timing of the responses, compared with directly injecting the peptides into the skin. It is possible that the delayed onset and prolonged vasodilation were due to the method of peptide delivery. Similar delays in the onset of vasodilation have been seen in our laboratory to the administration of another basic neuropeptide CGRP delivered via microdialysis (unpublished observations). Alternatively, vasodilation to histamine (a smaller amine), occurs rapidly (within ~2 min). Therefore, larger more basic peptides may diffuse from the microdialysis fibers more slowly increasing the time to the onset of vasodilation.

**Conclusions.** Cutaneous vasodilation to exogenous VIP includes a substantial NO-dependent portion in addition to a NO-independent component. Our results demonstrate that, although VIP-mediated vasodilation includes both a NO-dependent and H₁-activated portion, there does not appear to be a H₂-receptor component. Furthermore, the H₁-receptor component could not explain the entire NO-dependent portion. This suggests a histamine-independent NO portion to VIP-mediated vasodilation, possibly through direct stimulation by VIP. There does not appear to be any synergistic interaction between NO and VIP in human skin; however, our data cannot discount potential prejunctional interactions between NO and VIP (18).

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**REFERENCES**
