Vasoactive intestinal peptide fragment VIP₁₀₋₂₈ and active vasodilation in human skin

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Wilkins, Brad W., Brett J. Wong, Nathan J. Tublitz, Gregg R. McCord, and Christopher T. Minson. Vasoactive intestinal peptide fragment VIP₁₀₋₂₈ and active vasodilation in human skin. J Appl Physiol 99: 2294–2301, 2005. First published August 18, 2005; doi:10.1152/japplphysiol.00500.2005.—A recent study reported the vasoactive intestinal peptide (VIP) fragment VIP₁₀₋₂₈ inhibited the rise in skin blood flow during heat stress. Our laboratory has reported that the nitric oxide (NO) pathway and histamine receptor-1 (H1)-receptor activation is common to both exogenous VIP-mediated dilation and active vasodilation (AVD). The present study aimed to further examine the specific role for VIP in AVD by using VIP₁₀₋₂₈ to antagonize VIP-mediated dilation in the presence of NO synthase (NOS) inhibition and an H1 antagonist. Study 1 (n = 12) examined whether VIP₁₀₋₂₈ antagonizes vasodilation to exogenous VIP via inhibition of NO-dependent mechanisms. Study 2 (n = 6) investigated AVD in skin sites receiving VIP₁₀₋₂₈ alone and in combination with NOS inhibition. Study 3 (n = 6) examined AVD in sites receiving VIP₁₀₋₂₈ alone and combined VIP₁₀₋₂₈ and H1 antagonism. Due to differences in our findings and those previously published, study 4 (n = 6) investigated whether an increase in baseline skin blood flow could result in a diminished rise in AVD. Red blood cell flux was measured using laser Doppler flowmetry, and cutaneous vascular conductance (flux/mean arterial pressure) was normalized to maximal vasodilation (28 mM sodium nitroprusside). VIP₁₀₋₂₈ augmented vasodilation to exogenous VIP (P < 0.05 vs. control) and hyperthermia (P < 0.05 vs. control). NOS inhibition had no effect on the augmented dilation during exogenous VIP or hyperthermia (P > 0.05). Similarly, H1-receptor antagonists had no effect on the augmented dilation during hyperthermia (P > 0.05 vs. VIP₁₀₋₂₈). In study 4, percentage of maximal cutaneous vascular conductance was attenuated when baseline skin blood flow was elevated before whole body heating. Our results suggest that VIP₁₀₋₂₈ may be an unsuitable antagonist for examining a role for VIP-mediated dilation in human skin.

histamine receptor-1; cutaneous vascular conductance

THE INITIAL RISE IN SKIN BLOOD FLOW with increasing core temperature in humans exposed to passive whole body heating is due to withdrawal of sympathetic noradrenergic vasoconstrictor tone. Continued heat exposure elicits further increases in skin blood flow due to a sympathetic active vasodilator mechanism (7). This active vasodilation is believed to be associated with cholinergic nerves, where presynaptically inhibiting cutaneous cholinergic nerves abolishes both sweating and active vasodilation (12). The specific mechanisms underlying cutaneous active vasodilation remain elusive. Recent focus has been on the potential role of the neuropeptide VIP, which is known to be present in nerve fibers of human skin (6, 18, 20) and to be colocalized with acetylcholine in sympathetic cholinergic nerves in humans (19). To date, a study by Bennett et al. (1) using the VIP carboxyl-terminal fragment VIP₁₀₋₂₈ provides the most notable evidence of a role for VIP in cutaneous active vasodilation. Although these authors found that VIP₁₀₋₂₈ administration elicited vasodilation under baseline normothermic conditions, they demonstrated an attenuated rise in skin blood flow during hyperthermia from the elevated baseline in the presence of the fragment, suggesting the contribution of VIP to active vasodilation.

In addition to the potential role for VIP in thermoregulatory active vasodilation, several studies have shown that nitric oxide (NO) is necessary for full expression of active vasodilation, where NO synthase inhibition during passive whole body heating attenuates skin blood flow by ~30% (11, 22, 28). The specific source of this NO is unknown, although evidence suggests that acetylcholine may be a source of NO early in heating (24) but not as heating progresses (23). In the context of VIP-mediated vasodilation in human skin, we recently demonstrated a substantial NO-dependent component to vasodilation elicited by exogenous VIP administration under normothermic conditions (27). Therefore, VIP may be a potential source of NO production for active vasodilation in human skin. Recent evidence from our laboratory further suggests that histamine receptor-1 (H1) activation, with no activation of the histamine receptor-2 (H2) isoform, also contributes to cutaneous active vasodilation in humans (29). In light of our previous report that vasodilation to exogenous VIP administration includes a substantial H1 component without H2 activation (27), this finding further supports a role for VIP in active vasodilation. That is, the combination of the recent results published from our laboratory demonstrates that the characteristics of vasodilation to exogenous VIP and active vasodilation during hyperthermia are strikingly similar.

Using the above information as background, the present study initially aimed to investigate the specific role for VIP in thermoregulatory active vasodilation in human skin. We proposed to 1) examine whether VIP₁₀₋₂₈ antagonizes vasodilation to exogenous VIP in the skin via a NO-dependent mechanism, 2) examine the skin blood flow response during hyperthermia in skin sites receiving VIP₁₀₋₂₈ alone and when combined with NO synthase inhibition, and 3) examine vasodilation during hyperthermia in skin sites receiving VIP₁₀₋₂₈ alone and combined with a H1 antagonist. Our hypothesis was that cutaneous vascular conductance (CVC) in sites receiving NO synthase inhibition or H1 blockade alone would not differ from sites receiving VIP₁₀₋₂₈ alone or the combination of VIP₁₀₋₂₈ with either NO synthase inhibition or H1 antagonist.

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This finding would suggest a link between NO- or H1-dependent portions of active vasodilation and VIP. Due to differences in our findings and those of Bennett et al. (1), an additional study was designed to investigate whether raising baseline skin blood flow could account for the diminished rise in skin blood flow during hyperthermia previously observed in the presence of VIP10–28 (1).

METHODS

Subjects. Nineteen female (mean age 22 ± 1 yr) and 11 male (mean age 23 ± 1 yr) subjects volunteered to take part in four studies. 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. Female subjects were studied during the early follicular phase of the menstrual cycle or the placebo phase of oral contraceptives.

Instrumentation

Subjects were instrumented with an electrocardiogram monitored throughout each protocol with a CardioCap monitor (Datek-Ohmeda, Tewksbury, MA), and arterial pressure was assessed by brachial auscultation every 5 min. During whole body heating protocols, a water-perfused suit that covers the entire body except the face, hands, and area of skin being studied was used to control whole body skin temperature. This suit was covered with a water impermeable rain suit to limit evaporative heat loss. Whole body skin temperature was determined by the average of four copper-constantan thermocouples placed on the thigh, calf, abdomen, and chest. An index of core body temperature was measured continuously with a thermistor placed in the sublingual sulcus (T_{sub}).

Microdialysis fibers (MD 2000; Bioanalytical Systems, West Lafayette, IN) with 10-mm-long, 35-kDa cut-off membranes were placed in the skin of the ventral aspect of the nondominant forearm. The microdialysis probes were placed with a 25-gauge needle inserted through the dermis of the skin using sterile techniques in the absence of anaesthesia. 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). Skin 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 (MoorLAB, Moor Instruments) with integrated laser Doppler probes. After placement of the microdialysis fibers, skin blood flow was monitored to ensure that the insertion trauma had resolved before beginning each protocol (between 90 and 150 min).

Experiments

Presented in Fig. 1, A and B, is a schematic diagram of the general experimental design for studies 1–3.

**Study 1.** This general study design aimed to examine the ability of VIP10–28 alone and in combination with NO synthase inhibition to antagonize vasodilation during normothermic exogenous VIP administration. The peptide fragment VIP10–28 has been shown to competitively antagonize VIP receptors in some (8, 10, 13, 21) but not all tissues (15, 16, 25). To establish the efficacy of VIP10–28 to antagonize VIP-mediated vasodilation in human skin, six subjects were instrumented with four microdialysis fibers. Two microdialysis sites were randomly assigned to receive a continuous infusion of VIP10–28 (Sigma, St. Louis, MO) diluted in Ringer solution (200 μM infusion at 2 μl/min). The two remaining microdialysis sites received a continuous infusion of VIP10–28 together with N\(^\circ\)-nitro-\-arginine-methyl ester (\-NAME; CalBiochem, San Diego, CA) dissolved in Ringer solution (VIP10–28 and \-NAME; final concentrations of 200 μM VIP10–28 and 10 mM \-NAME). These infusions began 60 min before delivering a single dose of VIP (2 μl over 30 s) and continued throughout the remainder of the protocol. Based on our original characterization of VIP-mediated dilatation (27), 50- and 200-pmol doses of VIP (CalBiochem, San Diego, CA) were administered because the vasodilation to these doses were at the low (50 pmol) and high (200 pmol) end of the steep portion of the VIP dose response curve (27). Each dose of VIP was administered to a skin site that received the peptide fragment VIP10–28 and a site that received the VIP10–28 and \-NAME infusion. For comparison, four microdialysis sites in a second group of six subjects received the same 50- and 200-pmol doses of VIP (2 μl over 30 s) in skin sites receiving either Ringer solution alone or 10 mM \-NAME (continuous infusion at 2 μl/min) dissolved in Ringer solution (Fig. 1A). Red blood cell flux was monitored at each skin site during baseline, throughout VIP10–28 infusion, \-NAME infusion, VIP10–28 and \-NAME infusion, and until the established plateau following VIP administration. After the infusions, 28 mM sodium nitroprusside (SNP; Nitropress, Abbot Labs, Chicago, IL) was administered to maximally vasodilate the skin at each microdialysis site to enable the comparison of relative skin blood flow values (Fig. 1A). Because changing perfusion rate can affect the local environment near the membrane (2, 5), care was taken to ensure consistent perfusion rates across conditions.

**Study 2.** The aim of this study was to investigate the relative contribution of VIP and NO in thermoregulatory active vasodilation with VIP10–28 alone and in combination with NO synthase inhibition. Six subjects were instrumented with four microdialysis fibers where each microdialysis site was randomly assigned as a control site (site A) receiving Ringer solution throughout the normothermic and hyperthermic conditions, an experimental site (site B) receiving a continuous infusion of VIP10–28 (200 μM at 2 μl/min) throughout the normothermic and hyperthermic conditions, a second control site (site C) receiving a continuous infusion of \-NAME (10 mM at 2 μl/min) throughout each condition, and a second experimental site (site D) receiving VIP10–28 together with \-NAME (final concentrations of 200 μM VIP10–28 and 10 mM \-NAME at 2 μl/min) throughout each condition. Thermoneutral water (33°C) was perfused through the suit during the normothermic condition. Baseline measurements (15 min) preceded a continuous infusion (60 min) of VIP10–28 (site B), \-NAME (site C), or VIP10–28 and \-NAME (site D). The hyperthermic condition involved perfusing water through the suit at 50°C for ~60 min, sufficient to raise whole body skin temperature to 38 ± 0.2°C and T_{sub} to 0.8°C. After hyperthermia, 28 mM SNP was infused (4 μl/min) to maximally vasodilate the skin at each microdialysis site to enable the comparison of relative skin blood flow values (Fig. 1B).

**Study 3.** Skin blood flow responses to both exogenous VIP (27) and hyperthermia (29) have been shown to include an important H1-mediated vasodilation. Therefore, we designed a second study to investigate skin blood flow during hyperthermia in sites receiving a continuous infusion of VIP10–28 and the combination of VIP10–28 with the H1 antagonist pyrilamine. In this study, we doubled the VIP10–28 concentration (from 200 to 400 μM) to ensure that adequate drug delivery did not influence our results from studies 1 and 2.

Six subjects were instrumented with four microdialysis fibers where each microdialysis site was randomly assigned as follows: site A served as a control site and received Ringer solution throughout the normothermic and hyperthermic conditions; site B served as an experimental site receiving a continuous infusion of VIP10–28 (400 μM at 2 μl/min) throughout each condition; site C served as an experimental site receiving VIP10–28 together with pyrilamine (Sigma; final concentrations of 400 μM VIP10–28 and 500 μM pyrilamine at 2 μl/min) throughout each condition; and site D served...
as a second control site and received a continuous infusion of pyrilamine (500 μM at 2 μl/min) throughout each condition. We have previously demonstrated that a 500 μM dose of pyrilamine substantially attenuates cutaneous vasodilation to exogenous histamine (27) and cutaneous active vasodilation (29). The experimental design was similar to study 2, where baseline measurements (Ringer; 15 min) preceded a continuous infusion (60 min) of VIP10–28 (site B), L-NAME infusion (site C), and combined VIP10–28 and L-NAME infusion (site D). Skin sites in a single group (n = 6) received Ringer infusion (site A), VIP10–28 infusion (site B), L-NAME infusion (site C), and combined VIP10–28 and L-NAME infusion (site D). SNP, sodium nitroprusside.

**Study 4.** In the report by Bennett et al. (1), the VIP10–28 fragment caused vasodilation during a normothermic administration for 60 min before hyperthermia. In this study, we examined the possibility that increasing baseline skin blood flow before hyperthermia would attenuate the range by which skin blood flow can further increase due to active vasodilation. Six subjects were instrumented with two microdialysis fibers. Site 1 was a control site receiving a continuous Ringer infusion. Site 2 received a continuous infusion of SNP (2 μM at 4 μl/min) to increase skin blood flow before the onset of whole body heating. After the plateau in skin blood flow to continuous SNP infusion (site 2), subjects were exposed to whole body heating sufficient to raise Twb 0.8°C (~50 min). After hyperthermia, 28 mM SNP was infused (4 μl/min) to maximally vasodilate the skin at each microdialysis site to enable the comparison of relative skin blood flow values.
Data Acquisition and Analysis

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

Study 1. To examine the effect of VIP10–28 on baseline skin blood flow and to exogenous VIP-mediated dilation, CVC values were obtained during baseline Ringer infusion, after 1 h of continuous VIP10–28 infusion, and at the established plateau in skin blood flow after each dose of VIP. To determine whether VIP10–28 infusion alone affects skin blood flow, CVC values were compared between Ringer infusion and after 1 h of VIP10–28 by a paired t-test. To analyze the effect of VIP10–28 on VIP-mediated dilation, the plateau in CVC from each dose of VIP (50 and 200 pmol) were compared by a non-repeated-measures one-way ANOVA between VIP alone, VIP with VIP10–28 infusion, VIP with VIP10–28 and L-NAME infusion, and VIP with l-NAME infusion. Fisher’s least squared difference post hoc analysis was used to determine when significant differences occurred.

Study 2. To compare the skin blood flow response to the infusion of VIP10–28 under the normothermic condition, CVC values were obtained at each site at baseline, after 1 h of Ringer infusion (site A), VIP10–28 infusion (site B), L-NAME infusion (site C), and VIP10–28 and L-NAME infusion (site D). These values were compared at each site by one-way repeated-measures ANOVA. During the hyperthermic condition, CVC values were obtained in all microdialysis sites following a rise in Ttor of 0.8°C. The CVC values obtained during hyperthermia at each site were compared by one-way repeated-measures ANOVA. Fisher’s least squared difference post hoc analysis was used to determine when significant differences occurred.

Study 3. Similar to study 2 above, CVC values were obtained at each site after 60 min of Ringer infusion (site A), VIP10–28 infusion (site B), combined VIP10–28 and pyrilamine infusion (site C), and pyrilamine alone (site D). These values were compared at each site by one-way repeated-measures ANOVA. In the hyperthermic condition, CVC values were obtained in all microdialysis sites following a rise in Ttor of 0.8°C. The CVC values obtained during hyperthermia at each site were compared by one-way repeated-measures ANOVA. Fisher’s least squared difference post hoc analysis was used to determine when significant differences occurred.

Study 4. CVC values were obtained from each site at baseline, following the plateau in skin blood flow to 2 μM SNP infusion (site 2), and every 10 min during hyperthermia until a rise in Ttor of 0.08°C. The CVC responses during baseline, during 2 μM SNP infusion, and during hyperthermia were compared by one-way repeated-measures ANOVA. To compare the change in CVC (Δ%CVCmax) during hyperthermia, CVC values (expressed as %CVCmax) obtained from each site immediately before the onset of whole body heating (during the 2 μM SNP infusion in site 2) were subtracted from each value obtained during hyperthermia and were compared by one-way repeated-measures ANOVA. Fisher’s least squared difference post hoc analysis was used to determine when significant differences occurred. For all four studies, associated P values of <0.05 were considered statistically different. All values are presented as means ± SE.

RESULTS

Study 1

Figure 2 displays group data from administration of exogenous VIP with continuous VIP10–28 infusion and combined VIP10–28 and l-NAME infusion. These responses are compared with the same doses of exogenous VIP alone and VIP administered during NO synthase inhibition. The infusion of VIP10–28 (200 μM) for 1 h did not elicit vasodilation at any microdialysis site in any subject. However, administering a 50-pmol dose of VIP in the presence of VIP10–28 increased CVC to an established plateau statistically higher than the 50-pmol dose of VIP alone (80 ± 3 vs. 32 ± 5% CVCmax, respectively; P < 0.05). Similarly, the established plateau in CVC to a 200-pmol VIP dose with VIP10–28 infusion was statistically greater than the 200-pmol dose alone (87 ± 5 vs. 64 ± 4% CVCmax, respectively; P < 0.05: Fig. 2). The augmented dilation in the presence of VIP10–28 was not due to the enhanced production of NO, as there was no statistical difference in sites receiving VIP10–28 or VIP10–28 and l-NAME for either the 50- or 200-pmol VIP dose (P = 0.08 and 0.11, respectively; Fig. 2).

Study 2

Presented in Fig. 3 are the group data at each skin site after 60 min of infusion under the normothermic condition and following a rise in Ttor of 0.8°C during hyperthermia. Infusion
of VIP10–28 (site B), l-NAME (site C), or VIP10–28 and l-NAME (site D) did not affect skin blood flow under the normothermic condition compared with Ringer infusion in control sites (site A; \( P > 0.05 \); Fig. 3). Hyperthermia increased skin blood flow to 59 \( \pm 7\% \text{CVC}_{\text{max}} \) in control sites, 75 \( \pm 6\% \text{CVC}_{\text{max}} \) in sites receiving VIP10–28, 32 \( \pm 5\% \text{CVC}_{\text{max}} \) in sites receiving l-NAME, and 52 \( \pm 7\% \text{CVC}_{\text{max}} \) in sites receiving VIP10–28 and l-NAME. The increase in skin blood flow during hyperthermia was statistically greater in sites receiving a continuous infusion of VIP10–28 compared with the control site (\( P < 0.05 \); Fig. 3). The increase in skin blood flow during hyperthermia in skin sites receiving VIP10–28 and l-NAME was not statistically different from control (\( P = 0.20 \); Fig. 3). However, the combination of VIP10–28 and l-NAME substantially augmented vasodilation compared with l-NAME alone (\( P < 0.01 \); Fig. 3).

**Study 3**

Figure 4 displays group data from each skin site after 60 min of drug infusion under the normothermic condition and following a rise in \( T_{\text{or}} \) of 0.8°C during hyperthermia. Infusion of VIP10–28 (site B), VIP10–28 and pyrilamine (site C), or pyrilamine alone (site D) did not produce any dilation under the normothermic condition compared with Ringer infusion in the control site (site A; \( P > 0.05 \); Fig. 4). Hyperthermia increased skin blood flow to 62 \( \pm 8\% \text{CVC}_{\text{max}} \) in control sites, 69 \( \pm 4\% \text{CVC}_{\text{max}} \) in sites receiving VIP10–28, 68 \( \pm 9\% \text{CVC}_{\text{max}} \) in sites receiving VIP10–28 and pyrilamine, and 48 \( \pm 4\% \text{CVC}_{\text{max}} \) in sites receiving pyrilamine alone. Although the skin blood flow response during hyperthermia was not statistically greater in sites receiving VIP10–28 or sites receiving combined VIP10–28 and pyrilamine in this group of six subjects (\( P = 0.25 \) and \( P = 0.29 \) vs. control, respectively), the trends were similar to the results from study 1 demonstrating augmented active vasodilation in the presence of VIP10–28. In line with our laboratory’s previous findings (29), the H1 antagonist pyrilamine attenuated active vasodilation (\( P < 0.05 \) vs. control; Fig. 4).

**Study 4**

Presented in Fig. 5A are the group data from each skin site during baseline (normothermia), after the plateau in skin blood flow to continuous 2 μM SNP infusion (site 2), and during hyperthermia. Continuous infusion of 2 μM SNP increased CVC to 28 \( \pm 5\% \text{CVC}_{\text{max}} \) before hyperthermia (\( P < 0.001 \) vs. control). During hyperthermia, a rise in \( T_{\text{or}} \) of 0.8°C increased CVC to 58 \( \pm 5\% \text{CVC}_{\text{max}} \) in sites receiving continuous 2 μM SNP infusion and 57 \( \pm 4\% \text{CVC}_{\text{max}} \) in control sites (\( P = 0.92 \)). This suggests that predilation with SNP does not add to active vasodilation during hyperthermia. Figure 5B displays the change in CVC from each skin site immediately before the onset of body heating (time 0) and at 10-min intervals throughout hyperthermia. The rise in skin blood flow due to active vasodilation in skin sites receiving continuous 2 μM SNP was substantially less than control skin sites (\( P < 0.001 \)), suggesting that predilation of the cutaneous vasculature before whole body heating attenuates the range by which skin blood flow can further increase during hyperthermia.

**DISCUSSION**

Our findings indicate that, under every condition set in this series of studies, the administration of VIP10–28 did not appear to antagonize VIP-mediated vasodilation. This was the case during exogenous VIP administration and during hyperthermia. Therefore, we were not able to determine whether NO- and H1-dependent portions of active vasodilation were due to VIP. Furthermore, the augmented vasodilation observed in the presence of VIP10–28 was not explained by an enhanced NO-mediated dilatation or an enhanced H1-mediated dilation.

Inconsistencies in the antagonist properties of VIP10–28 have been discussed previously, where there may be large species differences and tissue heterogeneity in VIP receptor properties (14, 26). The VIP10–28 fragment can competitively antagonize VIP binding in isolated cell lines (9, 17, 26) and inhibit cAMP accumulation in isolated cells (26). In gastric smooth muscle strips (3, 8, 10) and gastric smooth muscle cell preparations...
VIP<sub>10–28</sub> has been shown to inhibit VIP-mediated relaxation. However, VIP<sub>10–28</sub> failed to antagonize VIP-mediated smooth muscle relaxation in isolated arteries (16, 25) and VIP-mediated dilation was not affected by the VIP<sub>10–28</sub> fragment in the rat thyroid (15). In fact, Michalkiewicz et al. (15) found that combined VIP and VIP<sub>10–28</sub> potentiates vasodilation in the rat thyroid compared with VIP alone, and Sejourne et al. (21) found that high concentrations of VIP<sub>10–28</sub> displayed agonist properties in a hamster model.

In the context of human skin, only one study has examined the blood flow response during hyperthermia in the presence of VIP<sub>10–28</sub> (1). These authors reported an attenuated change in skin blood flow (Δ%CVC<sub>max</sub>) during continuous exogenous VIP infusion. We were unable to confirm these findings when a single dose of exogenous VIP was administered to the skin and a plateau skin blood flow response to exogenous VIP was established (Fig. 2). The reason behind the different findings between our data and those of Bennett et al. (1) is unclear. It is possible that the difference lies in the method of exogenous VIP delivery. The presence of VIP<sub>10–28</sub> may affect the diffusion of VIP from microdialysis membranes. If this was the case, our data suggest that continuous infusion of VIP<sub>10–28</sub> facilitated the diffusion of VIP from the microdialysis membrane leading to the augmented cutaneous blood flow. This conclusion seems counterintuitive, where infusing high doses of VIP<sub>10–28</sub> should limit VIP diffusion. It seems entirely possible that VIP<sub>10–28</sub> administered together with the constant VIP infusion in the paper of Bennett et al. (1) could limit the number of molecules of VIP leaving the membrane, giving the appearance of antagonism by VIP<sub>10–28</sub>.

An important difference between the present investigation and that of Bennett et al. (1) is the finding that, in the latter study, VIP<sub>10–28</sub> elicited vasodilation before exogenous VIP infusion or hyperthermia (baseline blood flow). Bennett et al. (1) reported that 300 μM VIP<sub>10–28</sub> elicited maximal dilation, whereas in the present investigation 400 μM VIP<sub>10–28</sub> (study 3) did not cause vasodilation. This difference may be related to the properties of the membrane itself. That is, the microdialysis fiber manufacturers, either BioAnalytical Systems in the present investigation or Spectrum in the study of Bennett et al. (1), may use different materials for probe construction, which could affect diffusion from the membrane. Along these lines, our finding that VIP<sub>10–28</sub> augments vasodilation during hyperthermia, in addition to vasodilation to exogenous VIP, suggests that the diffusion of exogenous VIP from the microdialysis fiber was not the source but that the augmented vasodilation is due to some interaction occurring after diffusion from the membrane. There may also be slight differences in the dilution vehicle (Ringer) for VIP<sub>10–28</sub> solutions and/or any number of other subtle differences that may be responsible for the disparate results. Examining the subtle differences in methodology may become very important in the future when studies investigating the cutaneous circulation in humans are compared.

Bennett et al. (1) extended their findings from exogenous VIP delivery to include an attenuated rise in skin blood flow during hyperthermia in the presence of VIP<sub>10–28</sub>. However, these authors reported a maximal vasodilation to 300 μM VIP<sub>10–28</sub> and that VIP<sub>10–28</sub> at 214 μM limited the dilation to the antagonist. The degree of the vasodilation to the 214 μM infusion of VIP<sub>10–28</sub> was unclear in the report of Bennett et al. (1). The rise in skin blood flow during hyperthermia is presented only as a change in flow relative to prehyperthermia values. It is possible that under the conditions set in the report of Bennett et al. (1) VIP<sub>10–28</sub> activated vasodilator pathways during normothermia and thereby attenuated the range by which skin blood flow can increase during hyperthermia. This would manifest in a reduced rise in skin blood flow during hyperthermia. This was observed in study 4 of the present investigation. We increased skin blood flow under normothermic conditions with 2 μM SNP. The results indicate a reduced rise (Δ%CVC<sub>max</sub>) in skin blood flow during hyperthermia (Fig. 5B). That is, although hyperthermia increased skin blood flow in predilated sites to a similar CVC as in control sites (Fig. 5A), the further rise in CVC due to active vasodilation was blunted. If, in the report of Bennett et al. (1), they observed a significantly elevated baseline during VIP<sub>10–28</sub> infusion, the preceding discussion raises doubts about their conclusions that

Fig. 5. Group data from study 4. A: means ± SE CVC from control sites (filled bars) and sites receiving continuous 2 μM SNP (open bars). Values were obtained at baseline, after the plateau in skin blood flow to 2 μM SNP, and after a rise in T<sub>av</sub> of 0.8°C during hyperthermia. B: change in CVC (means ± SE) in control sites (●) and sites receiving continuous 2 μM SNP (○). Values were obtained at each 10-min interval during hyperthermia and are presented as change in CVC (Δ%CVC<sub>max</sub>) from prehyperthermia blood flow values. *P < 0.001 vs. control. †Main effect (P < 0.001) vs. control.
VIP\textsubscript{10–28} inhibited VIP-mediated vasodilation during hyperthermia. The result from study 4 in the present investigation offers an alternative explanation for their findings.

Another possible explanation for the discordant observations between our data and those of Bennett et al. (1) could be attributed to a greater amount of VIP\textsubscript{10–28} being diffused from microdialysis fibers in their study. However, the molecular mass cutoff of the microdialysis membranes used in their study was 18 kDa, with an effective molecular mass cutoff of \textasciitilde5 kDa, whereas the microdialysis membranes used in the present study had a molecular mass cutoff of \textasciitilde35 kDa with an effective cutoff of \textasciitilde7 kDa. It is therefore unlikely that the effective cutoff of microdialysis membranes could explain the differing result. When we doubled the concentration of VIP\textsubscript{10–28} in the perfusate in study 3 [400 vs. 214 \mu M in the report of Bennett et al. (1)], vasodilation during hyperthermia remained augmented. Our finding that VIP\textsubscript{10–28} augmented CVC in both the exogenous VIP-infusion study and the hyperthermia studies suggests that VIP\textsubscript{10–28} was leaving our fibers in sufficient quantity. Furthermore, the overall amount of VIP\textsubscript{10–28} infused (study 1) was over 200-fold higher than the 200-pmol exogenous VIP dose administered in the present report.

Despite our previous findings that cutaneous vasodilation to exogenous VIP includes a substantial NO-dependent component at doses above 100 pmol (27), the augmented vasodilation to exogenous VIP in the presence of VIP\textsubscript{10–28} was not due to an enhanced NO-dependent dilation (Fig. 2). Although not statistically different, the augmented dilation to exogenous VIP infusion appears to include an NO-dependent component. However, our finding that skin blood flow to exogenous VIP remained substantially augmented in sites receiving combined VIP\textsubscript{10–28} and NO synthase inhibition compared with NO synthase inhibition alone demonstrates that the augmented dilation was not due to an enhanced production of NO. Similarly, during whole body heating, our finding that active vasodilation was augmented in skin sites receiving the combination of VIP\textsubscript{10–28} and NO synthase, compared with NO synthase inhibition alone, suggests that there was no enhanced production of NO in VIP\textsubscript{10–28} sites during hyperthermia (Fig. 3). The reduction in skin blood flow with the addition of NO synthase inhibitor to VIP\textsubscript{10–28} during hyperthermia was similar to the reduction with NO synthase inhibitor alone. The interpretation of this finding is difficult but may suggest that the augmented dilation during VIP\textsubscript{10–28} infusion with hyperthermia is due to NO-independent mechanisms. In addition, the combination of VIP\textsubscript{10–28} with H1 antagonist augmented active vasodilation (Fig. 4), excluding enhanced H1 activation as a possible mechanism for the greater vasodilation in the presence of VIP\textsubscript{10–28}.

In conclusion, the results from this series of studies demonstrate that, under the conditions set in our laboratory, VIP\textsubscript{10–28} augments vasodilation to exogenous VIP administration and during hyperthermia. This augmented dilation is not due to an enhanced NO- or H1-mediated dilation during hyperthermia. In fact, the presence of VIP\textsubscript{10–28} fundamentally altered the ability of NO synthase inhibition and H1-antagonist to minimize dilation during hyperthermia. Lastly, the results from study 4 in the present investigation provide an alternative explanation for the findings of Bennett et al. (1).

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