Antagonism of soluble guanylyl cyclase attenuates cutaneous vasodilation during whole body heat stress and local warming in humans

Dean L. Kellogg Jr.,1,2,3 Joan L. Zhao,1,2 Yubo Wu,1,2 and John M. Johnson3

1Geriatric Research, Education, and Clinical Center, Department of Veterans Affairs, South Texas Veterans Health Care System, Audie L. Murphy Memorial Veterans Hospital Division, and 2Division of Geriatrics, Gerontology, and Palliative Medicine, Department of Medicine, and 3Department of Physiology, University of Texas Health Science Center at San Antonio, San Antonio, Texas

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Kellogg DL Jr, Zhao JL, Wu Y, Johnson JM. Antagonism of soluble guanylyl cyclase attenuates cutaneous vasodilation during whole body heat stress and local warming in humans. J Appl Physiol 110: 1406–1413, 2011. First published February 3, 2011; doi:10.1152/japplphysiol.00702.2010.—We hypothesized that nitric oxide activation of soluble guanylyl cyclase (sGC) participates in cutaneous vasodilation during whole body heat stress and local skin warming. We examined the effects of the sGC inhibitor, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), on reflex skin blood flow responses to whole body heat stress and on nonreflex responses to increased local skin temperature. Blood flow was monitored by laser-Doppler flowmetry, and blood pressure by Finapres to calculate cutaneous vascular conductance (CVC). Intradermal microdialysis was used to treat one site with 1 mM ODQ in 2% DMSO and Ringer, a second site with 2% DMSO in Ringer, and a third site received Ringer. In protocol 1, after a period of normothermia, whole body heat stress was induced. In protocol 2, local heating units warmed local skin temperature from 34 to 41°C to cause local vasodilation. In protocol 1, in normothermia, CVC did not differ among sites [ODQ, 15 ± 3% maximum CVC (CVCmax); DMSO, 14 ± 3% CVCmax; Ringer, 17 ± 6% CVCmax; P < 0.05]. During heat stress, ODQ attenuated CVC increases (ODQ, 54 ± 4% CVCmax; DMSO, 64 ± 4% CVCmax; Ringer, 63 ± 4% CVCmax; P < 0.05, ODQ vs. DMSO or Ringer). In protocol 2, at 34°C local temperature, CVC did not differ among sites [ODQ, 47 ± 2% CVCmax; DMSO, 18 ± 4% CVCmax; Ringer, 18 ± 3% CVCmax; P > 0.05]. ODQ attenuated CVC increases at 41°C local temperature (ODQ, 54 ± 5% CVCmax; DMSO, 86 ± 4% CVCmax; Ringer, 90 ± 2% CVCmax; P < 0.05 ODQ vs. DMSO or Ringer). sGC participates in neurogenic active vasodilation during heat stress and in the local response to direct skin warming.

nitric oxide; guanosine 3′,5′-cyclic monophosphate; microdialysis; skin; thermoregulation

IN HUMANS, THE CUTANEOUS CIRCULATION is a major effector of human thermoregulatory reflexes. During cold stress, reduced internal and skin temperatures (Tsk) lead to cutaneous vasoconstriction mediated by a sympathetic noradrenergic co-transmitter system, whereas, during heat stress, increases in these temperatures lead to reflex cutaneous vasodilatation mediated largely by a neurogenic cholinergic co-transmitter system (21). Under normothermic conditions, skin blood flow (SkBF) averages ~5% of cardiac output; however, the absolute flow of blood to the skin can vary from nearly zero during periods of cold stress to as much 8 l/min or 60% of cardiac output distributed over the body surface during maximal vasodilation in whole body heat stress (41).

The cutaneous active vasodilator system is responsible for 80–95% of the elevation in SkBF that accompanies heat stress (19, 42). Cutaneous active vasodilation, per se, is mediated by increased activity of sympathetic cholinergic nerves that release acetylcholine and one or more cotransmitters (6, 25). In addition to these “classical” neurotransmitters, nitric oxide (NO) production by NO synthase (NOS) is involved in the mechanism of cutaneous active vasodilation (22, 48). Approximately 30–45% of the increase in SkBF mediated by active vasodilation during heat stress is dependent on NO generation by NOS (22, 27, 47, 48, 57).

In addition to active vasodilation induced by whole body heating, local vasodilatory mechanisms respond to direct application of heat to skin, i.e., local warming can increase SkBF (21). The vasodilator response to local skin warming is biphasic, with sensory nerves mediating an initial transient vasodilatory “peak”, followed by a prolonged vasodilatory “plateau” that is mediated primarily by NOS generation of NO (24, 37) and has the potential of causing maximal vasodilation (53, 54).

While much work has addressed the role of NOS and NO generation in the control of SkBF, little work has examined how the NO generated mediates its vasodilator effects subsequent to its generation. Classically, the effects of NO are viewed as being dependent on activation of soluble guanylyl cyclase (sGC), although sGC-independent pathways for NO actions are extant (5, 33). NO-mediated activation of sGC is accomplished through the binding of the diatomic gas to the heme of sGC. Subsequent to activation, sGC effects the de-phosphorylation of guanosine 5′-triphosphate to yield increased levels of guanosine 3′,5′-monophosphate (cGMP) at a rate of up to 400-fold that of basal enzyme activity (11, 17). Increases in cGMP lead to activation of specific protein kinases, in particular cGMP-dependent protein kinase (protein kinase G), which cause decreases in intracellular Ca2+ levels and Ca2+ sensitivity and thereby mediate vascular relaxation (7, 17, 40).

While the physiological significance of the sGC/cGMP pathway for NO is well established, there is increasing evidence that NO can affect additional physiological signaling pathways by sGC/cGMP-independent mechanisms (14). sGC-independent pathways have been found in a number of blood vessel types in several species, including humans. Such pathways can be demonstrated by incomplete antagonism of vasodilatation by a selective sGC antagonist at doses that abolish NO-induced activation of sGC and cGMP production (56).
Material-induced by local skin warming. An analogous vasodilation evoked by whole body heat stress and on that hypothesis that NO mediates its vascular effects in cutaneous active vasodilation or local skin warming, or involved in transducing the effects of NO into increases in SkBF during cutaneous active vasodilation or local skin warming, or whether sGC/cGMP-independent mechanisms in either process are involved is unknown. We sought to test the hypothesis that NO mediates its vascular effects in cutaneous active vasodilation and local skin warming through activation of sGC-dependent mechanisms in humans. We tested that hypothesis by examining the effects of sGC inhibition on the cutaneous vasodilation evoked by whole body heat stress and on that induced by local skin warming.

Materials

Antagonism of sGC activation by NO was achieved by administration of 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ). SNP (50 μM) increased cutaneous vascular conductance (CVC) to 53 ± 5% maximum CVC at microdialysis sites perfused with Ringer solution and to 56 ± 5% maximum CVC at sites perfused with 2% DMSO (P < 0.01 vs. baseline sites). The addition of 1 mM ODQ dissolved in 2% DMSO solution attenuated this vasodilation to 22 ± 5% maximum CVC. Values are means ± SE, P < 0.01, ODQ vs. Ringer or 2% DMSO with SNP. P > 0.05 vs. Ringer or 2% DMSO baseline sites.

Whether the classical sGC-dependent mechanism is involved in transducing the effects of NO into increases in SkBF during cutaneous active vasodilation or local skin warming, or whether sGC/cGMP-independent mechanisms in either process are involved is unknown. We sought to test the hypothesis that NO mediates its vascular effects in cutaneous active vasodilation and local skin warming through activation of sGC-dependent mechanisms in humans. We tested that hypothesis by examining the effects of sGC inhibition on the cutaneous vasodilation evoked by whole body heat stress and on that induced by local skin warming.

Materials

Antagonism of sGC activation by NO was achieved by administration of 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), a potent and selective sGC antagonist that has been used to discriminate between cGMP-dependent and cGMP-independent effects of NO (11, 62). The agent competitively binds to the heme iron of sGC and inhibits NO-sensitive stimulation of cGMP production without altering basal sGC activity (31, 62). ODQ does not inhibit membrane-bound (particulate) guanylyl cyclases (62). Methylene blue and LY-83583 are also purported to be sGC antagonists; however, both of these agents additionally inhibit NO generation by NOS and enhance production of superoxide anion that can inactivate NO (1). These problematic aspects make methylene blue and LY-83583 unsuitable to define sGC-dependent and independent mechanisms and have led to the use of ODQ as the pharmacological agent of choice in defining such mechanisms (56).

Drug delivery was achieved by intradermal microdialysis to permit local administration of ODQ directly into the interstitial space of a small area of skin. This approach permitted monitoring of SkBF from untreated control areas and areas of high local drug concentration without potentially confounding systemic effects. SkBF was monitored by laser-Doppler flowmetry (LDF; MoorLab, Moor Instruments, Devon, UK) from the same small volume of skin (~1 mm³) over the microdialysis probes. LDF measurements are specific to skin, being uninfluenced by blood flow in the underlying skeletal muscle tissue (44).

A series of preliminary experiments was performed to determine the appropriate ODQ concentration to inhibit sGC maximally. In these studies, we used intradermal microdialysis and tested the ability of varying concentrations of ODQ to antagonize the vasodilatory effect of 50 μM sodium nitroprusside (SNP), an NO donor and activator of sGC (16, 55). This concentration of SNP was found to increase cutaneous vascular conductance (CVC) to approximately one-half of maximal levels, and a concentration of 1 mM ODQ attenuated this response significantly (see Fig. 1).

Because LDF measurements provide a relative index of SkBF, these measurements are often normalized to basal or maximal CVC to facilitate comparisons between or among treatment sites (20, 23). For microdialysis studies, perfusion with high concentrations of SNP is often used to achieve maximal vasodilation for data normalization (22, 37, 51). Because ODQ is a competitive antagonist of sGC, which is involved in effecting vasodilation by SNP, we verified that 58 mM SNP would overcome competitive antagonism of 1 mM ODQ. Preliminary experiments showed that perfusion with 1 mM ODQ did not attenuate the vasodilation caused by 58 mM SNP compared with sites that received no ODQ (see Table 1). This was further verified by our finding that local heating of the skin to 42°C, with or without reactive hyperemia [an NO-independent process in skin (35, 59, 61)] induced by 10 min of arm blood flow occlusion, added no further vasodilation to that achieved by 58 mM SNP at ODQ-treated sites. Based on these findings, we chose to normalize our CVC measurements to the maximal values achieved by perfusion of microdialysis probes with 58 mM SNP.

ODQ has limited aqueous solubility; therefore, the use of DMSO was required to increase the ODQ concentration achievable in Ringer solution. This approach has been used before in similar studies with other agents of poor aqueous solubility (29, 46). A concentration of 1 mM ODQ could be achieved in a solution of 2% DMSO in Ringer. Greater ODQ concentrations could only be achieved with higher concentrations of DMSO (2.5 mM ODQ required 10% DMSO in Ringer), but provided no greater attenuation of vasodilatory responses to SNP. Based on the foregoing findings and to avoid any unanticipated effects of DMSO, we chose to use 1 mM ODQ in 2% DMSO in Ringer for our subsequent studies, because this concentration maximally inhibited the sGC-mediated vasodilation induced by 50 μM SNP, yet required a minimal concentration of DMSO. In the study protocols, one microdialysis site was perfused with 2% DMSO in Ringer solution to serve as a control for any effects of this agent (46).

Microdialysis probes were made in our laboratory from polyimide tubing and a 1-cm length of capillary microdialysis membrane (200-μm diameter, molecular cutoff 20 kDa) reinforced by a 51-μm-diameter coated stainless steel wire placed in the lumen of the membrane and tubing. On arrival in the laboratory, each subject had three probes placed at separate sites on the ventral aspect of one forearm as follows. Ice was applied to the chosen skin site to achieve baseline CVC 5.5 ± 0.9, 5.3 ± 0.6, 5.4 ± 0.8. Maximum CVC (58 mM SNP) 44.1 ± 7.1, 45.2 ± 5.9, 46.4 ± 6.2.

Values are means ± SE in arbitrary units. ODQ, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one; CVC, cutaneous vascular conductance; SNP, sodium nitroprusside.

Table 1. Absolute values of baseline and maximal cutaneous vascular conductance in arbitrary units

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<th>Ringer</th>
<th>2% DMSO</th>
<th>1 mM ODQ</th>
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<tr>
<td>Baseline CVC</td>
<td>5.5 ± 0.9</td>
<td>5.3 ± 0.6</td>
<td>5.4 ± 0.8</td>
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<tr>
<td>Maximum CVC (58 mM SNP)</td>
<td>44.1 ± 7.1</td>
<td>45.2 ± 5.9</td>
<td>46.4 ± 6.2</td>
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Fig. 1. Attenuation of sodium nitroprusside (SNP) induced vasodilation by 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ). SNP (50 μM) increased cutaneous vascular conductance (CVC) to 53 ± 5% maximum CVC at microdialysis sites perfused with Ringer solution and to 56 ± 5% maximum CVC at sites perfused with 2% DMSO (P < 0.01 vs. baseline sites). The addition of 1 mM ODQ dissolved in 2% DMSO solution attenuated this vasodilation to 22 ± 5% maximum CVC. Values are means ± SE. P < 0.01, ODQ vs. Ringer or 2% DMSO with SNP. P > 0.05 vs. Ringer or 2% DMSO baseline sites.

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The subjects’ average age (±SE) was 39 ± 5 years. Seven healthy subjects (3 men and 4 women) participated in this part of the study. The subjects’ average age (±SE) was 39 ± 3 years, average weight was 61 ± 4 kg, and average height was 161 ± 2 cm. To induce thermoregulatory reflexes, subjects wore a tube-lined suit. The suit was used to control Tsk by perfusion with water of different temperatures to produce periods of normothermia, cold stress, and heat stress (43). The suit was perfused with cold water to decrease Tsk and induce cold stress and warm water to raise Tsk to 38–39°C during heating periods. Over the suit, subjects wore a water-impermeable plastic garment to insulate them from the environment and prevent sweat evaporation. The suit and garment covered the entire body, with the exception of the head, hands, feet, and the forearm, from which blood flow measurements were made.

Internal temperature was monitored with a thermocouple held in the sublingual sulcus [oral temperature (Toral)]. Tsk was recorded as the weighted electrical average from six thermocouples taped on the skin surface (18, 52). Pulse rate (PR) and mean arterial pressure (MAP) were recorded continuously from a finger (Finapres BP Monitor, Ohmeda, Madison, WI) (39).

For the study, subjects were placed in the supine position and instrumented. Tsk was maintained at 34°C. Data collection began 2 h following microdialysis probe placement with a 5- to 10-min baseline control period during which the microdialysis probes were perfused with Ringer solution at a rate of 2 μl/min using a micro-infusion pump. After this control period, the perfusate at one site was changed to 2% DMSO in Ringer and to 1 mM ODQ in the 2% DMSO-Ringer solution at another site. Perfusion was maintained with Ringer only at the third site. Perfusion was maintained with these solutions for 45 min under normothermic conditions to allow ODQ to reach steady state in the intradermal space. Following this normothermic period, Tsk was decreased to induce cold stress for 3 min. Tsk was then raised to 38–39°C and maintained at that level for 35–50 min to induce heat stress and thereby stimulate the active vasodilator system. Body heating was maintained until LDF values at all sites had achieved stable elevated plateaus. Subjects were then cooled and returned to a normothermic Tsk of 34°C. All microdialysis sites were then perfused with 58 mM SNP to effect maximal vasodilation at each site. CVC values were normalized to these maximal levels for data analysis. The protocol is illustrated in Fig. 2.

Data are presented as means ± SE. For data analysis, CVC values were indexed as LDF/MAP and were normalized to their respective maxima, as elicited by 58 mM SNP to facilitate comparisons among sites, both within and among subjects, after verifying that there were no statistically significant differences among the absolute values achieved with SNP perfusion (P > 0.05 among sites). The vasomotor responses at the different microdialysis sites were analyzed by comparing the Ttor thresholds at which CVC increases started during whole body heating. The internal temperature threshold for the onset of vasodilation for each site was defined as the level of Ttor at which a sustained increase in CVC began during whole body heating and were chosen from graphs of CVC vs. Ttor by an investigator blinded as to the conditions, subjects, and antagonist treatment. The thresholds for cutaneous vasodilation were compared by ANOVA for repeated measures. Normothermic baseline CVC values, CVC levels during the final minute of drug infusion in normothermia, CVC during the final minute of cold stress, and CVC from the final 3 min of heat stress were also compared among sites by ANOVA for repeated measures, followed by specific means comparisons. MAP and PR changes from normothermia to the end of heat stress were compared by paired t-tests. The level of statistical significance was defined as P < 0.05.

Protocol 2: local skin warming. Seven healthy subjects (3 men and 4 women) participated in this part of the study. The subjects’ average age (±SE) was 33 ± 6 yr, average weight 61 ± 3 kg, and average height 161 ± 3 cm.

Subjects were placed in the supine position and instrumented to measure LDF at all microdialysis sites. Each LDF probe was equipped with a special holder that incorporated both heating elements and thermocouples to permit simultaneous LDF measurements and control of local skin temperature (Tloc) (24). A Finapres device was used for continuous monitoring of PR and MAP.

Data collection began with a 5- to 10-min control period with Tloc maintained at 34°C. Subsequently, the perfusate of one microdialysis site was maintained with Ringer solution, whereas the perfusate at a second microdialysis site was changed to 1 mM ODQ in 2% DMSO/Ringer solution. The perfusate at a third microdialysis site was changed to 2% DMSO in Ringer solution. Perfusion rate at all sites was 2 μl/min. Tloc was maintained at 34°C for 45 min, after which Tloc was increased slowly over a period of 20 min to 41°C at all sites to evoke vasodilation. A slow increase in temperature to 41°C was chosen to

![Fig. 2. Whole body heat stress protocol. This protocol was designed to examine the effects of antagonism of soluble guanylyl cyclase by ODQ on the reflex vasodilation induced by whole body heat stress. Three intradermal microdialysis sites were perfused with either 1 mM ODQ in 2% DMSO, 2% DMSO, or Ringer solution alone. Maximal cutaneous vasodilation was achieved at all sites at the end of the study by perfusion with 58 mM SNP. The perfusion rate at all microdialysis sites was 2 μl/min. AVD, active vasodilator.](http://jap.physiology.org/)

![Fig. 3. Local skin warming protocol. This protocol was designed to examine the effects of antagonism of soluble guanylyl cyclase by ODQ on the vasodilation induced by local warming of the skin. Three intradermal microdialysis sites were perfused with 1 mM ODQ in 2% DMSO, 2% DMSO, or Ringer solution. Maximal cutaneous vasodilation was achieved at all sites at the end of the study by perfusion with 58 mM SNP. The perfusion rate at all microdialysis sites was 2 μl/min. Tloc, local skin temperature.](http://jap.physiology.org/)
avoid pain fiber activation, which evokes skin vasodilation by NO-independent mechanisms (24). Finally, the perfusates at all sites were changed to 58 mM SNP for data normalization (26, 32). The protocol is illustrated in Fig. 3.

Data are presented as means ± SE. For data analysis, CVC was calculated (CVC = LDF/MAP) and normalized to maximum for each site (22). Normalized CVC responses were analyzed by comparing the mean levels achieved during initial peaks (when observed) and the final 3 min of the two thermal periods by repeated-measures ANOVA, with the level of statistical significance defined as 0.05.

RESULTS

Protocol 1: whole body heat stress. A representative result from one subject is illustrated in Fig. 4. CVC responses at each thermal condition of protocol 1 are summarized in Fig. 5. Under normothermic conditions, during perfusion of all microdialysis sites with Ringer solution only, there were no significant differences for the entire group among CVC values at the different sites (P > 0.05). CVC values in normothermia were unaltered when the perfusate at one site was changed to 1 mM ODQ in 2% DMSO and to 2% DMSO at a second site (P > 0.05 among sites). In response to cold stress, CVC fell at all sites (P < 0.05 vs. normothermia). These responses did not differ among treatment sites (P > 0.05 among sites).

At the peak of heat stress, CVC at sites perfused with Ringer reached 63 ± 4% maximum and 64 ± 4% maximum at sites that received 2% DMSO. These values did not significantly differ (P > 0.05). CVC at sites perfused with ODQ reached 54 ± 4% maximum, a value that was significantly less than those at the other two sites (P < 0.05).

During whole body heating, CVC at the sites perfused with Ringer began to rise when T or reached values of 36.9 ± 0.1°C, at 36.9 ± 0.1°C for sites perfused with ODQ, and at 36.9 ± 0.1°C for sites perfused with 2% DMSO. There was no statistical difference among these T or threshold values (P > 0.05).

Under normothermic conditions, MAP averaged 85 ± 3 mmHg and fell to 78 ± 4 mmHg at the peak of heat stress (P < 0.05, see Fig. 5). PR averaged 60 ± 2 beats/min in normothermia and increased to 93 ± 3 beats/min at the peak of heat stress (P > 0.01). T or averaged 36.9 ± 0.1°C in normothermia and increased to 37.6 ± 0.1°C at the end of heat stress (P < 0.01).

Protocol 2: local skin warming. A representative result from one subject is illustrated in Fig. 6. During perfusion with Ringer only, there were no statistical differences in CVC among sites (P > 0.05). At T loc = 34°C, CVC averaged 18 ± 3% maximum at untreated sites, 18 ± 3% maximum at sites that received 2% DMSO, and 14 ± 2% maximum at sites that were treated with 1 mM ODQ. These CVC values did not differ significantly among sites (P > 0.05 among sites).

When T loc was slowly increased to 41°C, small initial peaks were observed in four of seven subjects and did not differ...
among sites ($P > 0.05$). Thereafter, CVC at all sites increased significantly to stable plateau levels ($P < 0.05$ vs. $34^\circ C$). These CVC values were $90 \pm 2\%$ maximum at untreated sites, $86 \pm 4\%$ maximum at $2\%$ DMSO sites, and $54 \pm 5\%$ maximum at sites that were treated with $1\ mM$ ODQ. The CVC values at sites treated with ODQ were attenuated compared with those attained at untreated and $2\%$ DMSO sites ($P < 0.05$, ODQ vs. untreated or DMSO). CVC increases at untreated and DMSO-treated sites did not differ ($P > 0.05$ between sites). These results are summarized in Fig. 7.

**DISCUSSION**

The important new findings of our study are that the specific sGC antagonist ODQ attenuated the increases in CVC induced by the cutaneous active vasodilator system during whole body heat stress and the nonreflex cutaneous vasodilator response to local skin warming compared with responses at sites perfused with Ringer only or perfused with $2\%$ DMSO. Responses at sites perfused with $2\%$ DMSO did not differ from those perfused with Ringer only, showing that control responses were not due to DMSO. Our findings show that the classical pathway of heme-dependent activation of sGC by NO effects at least part of the vasodilator responses to both whole body heat stress and to local skin warming.

While the above result was not unexpected, the relatively small degree to which ODQ attenuated cutaneous active vasodilation relative to the vasodilation induced by local warming was unanticipated. Based on studies with NOS antagonists, it is generally accepted that $\sim 30$–$45\%$ of the increase in SkBF mediated by active vasodilation during heat stress is dependent on NO generation by NOS (22, 27, 47, 48, 57). The remainder of the overall active vasodilator response is presumably through vasoactive intestinal peptide or other cotransmitters acting through pathways independent of NO (6, 34, 47, 58, 60). If NO acted to effect cutaneous active vasodilation solely through the NO-sGC-cGMP pathway, a similar amount should be due to activation of sGC. Based on the present findings with sGC antagonism, only $\sim 15\%$ of the increase in SkBF during heat stress was attributable to sGC activation and suggests the novel conclusion that a significant portion of the heat stress-induced active cutaneous vasodilation effected by NO is not mediated by sGC-dependent mechanisms, but rather by sGC-independent mechanisms.

During the initiation of responses to heat stress, we found that the $T_{tor}$ threshold for the initiation of active vasodilation was not altered by sGC antagonism. If NO activation of sGC were a determinant of the threshold for vasodilation, the threshold would have been altered by ODQ, but this was not the case. This lack of effect is consistent with the lack of effect reported with many (10, 22, 29, 49), but not all (60), NOS antagonist-based studies. Our observation that sGC antagonism does not alter the $T_{tor}$ threshold for vasodilation reinforces the view that the threshold for initiation of active vasodilation is not dependent on the generation of NO by the NOS system, but instead is more likely dependent on the vesicular release of classical neurotransmitters (28, 29).
In contrast to the relatively small attenuation of cutaneous active vasodilation during whole body heat stress, antagonism of sGC by 1 mM ODQ caused a much greater attenuation of the vasodilation induced by local warming. Compared with the responses at sites perfused with 2% DMSO, 1 mM ODQ attenuated the local warming vasodilation by 37 ± 6%, in contrast to the 15 ± 5% attenuation during whole body heat stress. This suggests a greater role for the NO-sGC-cGMP pathway in the vasodilator response to local heating. These results are consistent with studies with NOS antagonists that show ~50% of the increase in SkBF during local skin warming to be dependent on NOS generation of NO (28, 30, 37, 50).

ODQ, the antagonist used in our study, is highly selective for NO-sensitive sGC, which it inhibits by oxidizing the ferrous form of the enzyme’s heme to the ferric form that has poor NO sensitivity, thus antagonizing heme-dependent activation of sGC by NO (12, 45). ODQ was chosen because of its higher specificity for sGC relative to methylene blue or LY-83538, which also inhibit sGC, but which also inhibit NOS and generate superoxide (16). ODQ does not inhibit particulate guanylyl cyclases, which are stimulated by peptides, such as atrial natriuretic peptide (62), nor does it inhibit adenyl cyclases (11). ODQ has been found to have an in vitro IC₅₀ as low as 50 nM in studies in which sGC activity was stimulated with SNP, and that 10 μM concentrations achieve maximal inhibition of sGC (56). ODQ is thus an important tool that has been used extensively to successfully elucidate roles of NO-sensitive sGC in a wide variety of physiological processes (11, 13, 62).

ODQ did not alter SkBF under normothermic conditions, nor did this agent alter the cutaneous vasoconstrictor response to cold stress relative to responses at sites that were perfused with Ringer or 2% DMSO in Ringer. This result shows that ODQ did not alter the vasoconstrictor system and supports the assumption of specificity of ODQ for sGC inhibition in our protocol.

SNP is a NO donor and causes vasodilation largely through heme-dependent activation of sGC (16, 55). The concentration of 1 mM ODQ used in our protocols produced the same inhibition of SNP-induced, sGC-dependent vasodilation that we observed in preliminary studies with ODQ concentrations of up to 2.5 mM. This result indicates that maximal inhibition of heme-dependent NO activation of sGC was achieved with 1 mM ODQ; however, we cannot exclude the possibility that 1 mM ODQ achieved only partial blockade.

While sGC has usually been viewed as the mediator of NO vasodilator effects, NO can also effect physiological signaling through sGC-independent mechanisms, such as S-nitrosation (14, 64). This sGC-independent process involves posttranslational modification of proteins at the thiol side chains of cysteine residues directly by NO and creates an S-nitrosothiol modification of the thiol side chain. Protein S-nitrosation is a reversible covalent modification and can affect proteins of all types in a fashion analogous to protein phosphorylation or acetylation (63). This process generally occurs at higher concentrations of NO than required for sGC activation alone and also occurs on a longer time course of up to a few minutes rather than the seconds typically required for sGC activation (2, 13, 64). S-nitrosation of proteins is involved in the modulation of neurotransmitter release (36, 38). NO-mediated S-nitrosation specifically facilitates soluble N-ethylmaleimide-sensitive factor attachment protein receptor complex formation and subsequent exocytosis (9, 38). Our findings clearly show a role for heme-dependent sGC activation by NO in the control of skin blood vessels and raise the possibility that S-nitrosation could also play a role. Involvement of such a mechanism in cutaneous active vasodilation would be consistent with the finding by Wilkins et al. (57) that exogenous NO caused greater vasodilation during heat stress than in normothermia, and their proposal that NO interacts with other neurotransmitters to synergistically augment cutaneous active vasodilation during heat stress. Given that we cannot totally exclude incomplete sGC antagonism by 1 mM ODQ in our studies, establishing such a role for S-nitration will require additional studies.

In contrast to our findings with whole body heat stress, our results show that the NO portion of the vasodilation due to local skin warming is mainly through heme-dependent sGC activation, and that the roles for other mechanisms are minor. Because increases in SkBF effected by local warming appear to be mainly due to NO binding to sGC heme to cause activation, our results support the clinical use of local skin warming to assess this NO-mediated sGC aspect of endothelial function (8).

In summary, we found that specific and maximal antagonism of sGC with ODQ attenuated both cutaneous active vasodilation in heat stress and the vasodilation induced by local skin warming. These results indicate that NO-sensitive, heme-dependent sGC activation is involved in both vasodilator responses. In addition, our results suggest that NO mediates cutaneous active vasodilation in heat stress both by sGC-independent mechanisms, as well as by sGC-dependent mechanisms, in approximately equal amounts. Our finding that ODQ antagonism of sGC activation had a much greater effect on the vasodilation induced by local skin warming suggests that NO mediation of the local warming response is more reliant on heme-dependent sGC activation.

GRANTS
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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

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SOLUBLE GUANYLYL CYCLASE AND CUTANEOUS VASODILATION


