Minimal role for H1 and H2 histamine receptors in cutaneous thermal hyperemia to local heating in humans

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Wong, Brett J., Sarah J. Williams, and Christopher T. Minson. Minimal role for H1 and H2 histamine receptors in cutaneous thermal hyperemia to local heating in humans. J Appl Physiol 100: 535–540, 2006. First published September 29, 2005; doi:10.1152/japplphysiol.00902.2005.—The precise mechanism(s) underlying the thermal hyperemic response to local heating of human skin are not fully understood. The purpose of this study was to investigate a potential role for H1 and H2 histamine-receptor activation in this response. Two groups of six subjects participated in two separate protocols and were instrumented with three microdialysis fibers on the ventral forearm. In both protocols, sites were randomly assigned to receive one of three treatments. In protocol 1, sites received 1) 500 μM pyrilamine maleate (H1-receptor antagonist), 2) 10 mM L-NAME to inhibit nitric oxide synthase, and 3) 500 μM pyrilamine with 10 mM Nω-nitro-L-arginine methyl ester (L-NAME). In protocol 2, sites received 1) 2 mM cimetidine (H2 antagonist), 2) 10 mM L-NAME, and 3) 2 mM cimetidine with 10 mM L-NAME. A fourth site served as a control site (no microdialysis fiber). Skin sites were locally heated from a baseline of 33 to 42°C at a rate of 0.5°C/5 s, and skin blood flow was monitored using laser-Doppler flowmetry (LDF). Cutaneous vascular conductance was calculated as LDF/mean arterial pressure. To normalize skin blood flow to maximal vasodilation, microdialysis sites were perfused with 28 mM sodium nitroprusside, and control sites were heated to 43°C. In both H1 and H2 antagonist studies, no differences in initial peak or secondary plateau phase were observed between control and histamine-receptor antagonist sites, but there was no effect of H2 antagonist on the nadir response. These data suggest only a modest role for H1-receptor activation in the cutaneous response to local heating as evidenced by a diminished nadir response and no role for H2-receptor activation.

microdialysis; cutaneous circulation; nitric oxide; neuropeptides

RAPID, NONPAINFUL LOCAL HEATING of human skin results in a large and sustained increase in skin blood flow. This thermal hyperemia to locally applied heat is characterized by two distinct phases. Within minutes of direct application of heat, there is a rapid and transient increase in skin blood flow, termed an initial peak and nadir response, which is followed by a more prolonged secondary plateau phase. The initial peak is thought to be an axon reflex-mediated response, whereas the secondary plateau is mediated mainly by nitric oxide (NO) (25, 28, 29). An important caveat is the secondary plateau is rendered insensitive to NO synthase inhibition if the local heating results in even brief periods of pain, suggesting there are different mechanisms mediating a nonpainful heating stimulus and a painful stimulus (25). To date, the precise mechanism(s) mediating the thermal hyperemic response to direct, rapid, nonpainful local heat remain unresolved, due in part to the complex interaction between locally produced vasodilators such as NO and neurally mediated vasodilation.

It has been suggested the axon reflex-mediated initial peak and nadir responses are due to activation of C-fiber afferent nerves that release the neuropeptides substance P and calcitonin gene-related peptide (CGRP), both of which have been found to be localized in nerve terminals in human skin (28, 29, 37, 38). In human skin, these peptides have been shown to induce vasodilation via multiple, and possibly redundant, pathways. Substance P and CGRP have been shown to induce release of NO from dermal endothelial cells, and vasodilation to both peptides is inhibited by an NO synthase inhibitor (9, 16, 26, 44). In this context, our laboratory has previously observed an attenuated initial peak and nadir response when an NO synthase inhibitor was administered during local heating of the skin. In the same study, the initial peak and nadir response, but not the secondary plateau, were further reduced when axon reflexes were blocked with EMLA cream (2.5% lidocaine and 2.4% prilocaine; Ref. 29). Additionally, it has been shown that the secondary plateau phase is predominantly mediated by local production of NO, where NO synthase inhibition during the plateau phase reduces skin blood flow to near-baseline values, suggesting NO plays a large role but is not the sole local vasodilator (25, 29).

Histamine and histamine-receptor activation could potentially contribute to the thermal hyperemic response. The H1 isoform of the histamine receptor has been shown to be localized on endothelial cells, and histamine exerts its vasodilatory effects by binding to H1 receptors and increasing production of local vasodilators such as NO (8, 21). Similarly, H2 receptors have been shown to exist in the cutaneous vasculature of humans (17, 18), and vasodilation to exogenous histamine has been shown to be attenuated in the presence of an H2-receptor antagonist (17–20, 24, 43).

In human skin, it has been shown that an intradermal injection of histamine results in an increase in NO production and contributes to a portion of histamine-mediated vasodilation (10, 11). Along these lines, substance P, but not CGRP, has been shown to degranulate human cutaneous mast cells and to increase the concentration of histamine in human skin, suggesting a portion of substance P-mediated vasodilation in human skin may be due to histamine and histamine receptor activation (5, 10, 14, 16, 23, 27, 30–32, 37). Thus, if substance
P is involved in thermal hyperemia, it is possible that heat-induced vasodilation includes an H1- and/or H2-receptor component, and it is possible that there is an interaction between histamine-receptor activation and NO.

Over the last several years, the use of local skin heating has emerged as a noninvasive clinical tool for assessing microvascular, and possibly endothelial, function in various patient populations, such as diabetes, chronic renal failure, systemic sclerosis, and Raynaud’s phenomenon (1, 4, 35, 36, 41). However, because the basic mechanisms underlying the thermal hyperemic response to local skin heating remain unclear, it is of importance to further understand the mechanisms in healthy subjects.

The purpose of this study was to investigate the role of H1 and H2 histamine receptors in the thermal hyperemic response to local heating of the skin and to determine whether a portion of the NO component could be explained by histamine-receptor activation. Our laboratory has previously reported that cutaneous active vasodilation and vasoactive intestinal peptide (VIP)-mediated vasodilation are attenuated in the presence of an H1-, but not an H2-, receptor antagonist (40, 43). Therefore, we tested the hypothesis that both the initial peak and secondary (NO dependent) plateau of the local heating response would be attenuated in the presence of an H1-, but not an H2-, receptor antagonist.

**METHODS**

**Subjects.** Seven women (22 ± 2 yr) and five men (25 ± 3 yr) participated in two separate, parallel protocols designed to examine the contribution of H1 (protocol 1) and H2 (protocol 2) histamine-receptor activation in cutaneous thermal hyperemia to local heating. Six subjects (4 women and 2 men) participated in protocol 1, and six subjects (3 women and 3 men) participated in protocol 2. Before participation, each subject gave written, informed consent, and this study was approved by the Institutional Review Board of the University of Oregon. All subjects were healthy, were normotensive, did not smoke, did not have diabetes, were normally active (did not exercise >1 h/day, 4 days/wk), and were not on any medications. All female subjects were studied in the early follicular phase of the menstrual cycle with the exception of one subject. Because the data for this one female subject did not differ, the data were grouped for subsequent analysis.

**Instrumentation.** For each protocol, subjects were in the supine position with the experimental arm at heart level. Subjects’ blood pressure was measured every 5 min via automated brachial auscultation (CardioCap, Datex-Ohmeda, Tewksbury, MA).

Subjects were instrumented with three microdialysis fibers (MD 2000, Bioanalytical Systems, West Lafayette, IN) on the ventral surface of the forearm. The membrane of the microdialysis fibers were 10 mm in length with a 20-kDa molecular mass cutoff. A 25-gauge needle was inserted into the dermal layer of the skin. The microdialysis fiber was then threaded through the lumen of the needle, and the needle and microdialysis fiber were pulled through the skin, leaving the membrane in place. Before each protocol, the initial trauma hyperemia associated with placing microdialysis fibers was allowed to subside (~90–120 min). During the trauma resolution, lactated Ringer solution was perfused through each fiber at a rate of 2 μl/min via a microinfusion pump (CMA/102, CMA Microdialysis, Stockholm, Sweden). A fourth site without a microdialysis fiber served as a control site in each protocol and was placed on a forearm location away from the microdialysis sites so as to avoid interaction with any of the experimental drugs. Pilot studies from our laboratory and data from Kellogg and colleagues (25) have shown that the thermal hyperemic response to rapid, nonpainful local heating is not affected by placement of microdialysis fibers in the skin.

To obtain an index of skin blood flow, red blood cell (RBC) flux was monitored via laser-Doppler flowmetry (MoorLAB, Moor Instruments, Devon, UK). Local heating devices (SH02 Skin Heaters, Moor Instruments) were used to control skin temperature and were placed directly over each microdialysis membrane. Integrated laser-Doppler probes designed to fit in the local heating units were used to measure RBC flux directly over each microdialysis membrane.

**Local heating protocol.** After the insertion trauma, the local heating devices were turned on and held constant at 33°C for 10 min during baseline data collection. After the baseline period, the temperature of the local heating devices was increased at a rate of 0.5°C every 5 s to a temperature of 42°C. This rate of local heating results in an increase in skin temperature to ~40°C and typically does not result in any sensation of pain (29). No subject reported any sensation of pain during the local heating period. The local heaters were held constant at 42°C until skin blood flow reached a stable 10- to 15-min plateau.

**Protocol 1: H1-receptor antagonist studies.** The purpose of this protocol was to determine the contribution of H1-receptor activation to the skin blood flow response to local heating. Six subjects (4 women and 2 men) participated in this protocol. Subjects were instrumented with microdialysis fibers, local heaters, and laser-Doppler probes as described above. The first microdialysis site was perfused with 500 μM of pyrilamine maleate, an H1 receptor antagonist (Sigma, St. Louis, MO). Our laboratory has shown previously that this dose of pyrilamine in human skin significantly attenuates the vasodilation to exogenous histamine and VIP as well as during whole body heat stress (40, 43). The second microdialysis site was perfused with 10 mM of the l-arginine analog Nω-nitro-l-arginine methyl ester (l-NAME; Calbiochem, San Diego, CA) to inhibit NO synthase. It has been previously shown that this concentration of l-NAME adequately inhibits NO synthase in human skin (25, 29). The third microdialysis site was perfused with 500 μM pyrilamine and 10 mM l-NAME (final concentrations) and was used to examine any potential interaction between H1-receptor activation and NO during local skin heating. A fourth site without a microdialysis fiber served as a control site.

All drugs were perfused through the microdialysis fibers with a microinfusion pump at a rate of 2 μl/min for 30–45 min before the local heating protocol and were continuous for the duration of the local heating. The local heaters were then increased from 33 to 42°C as described above. Once a stable plateau in skin blood flow was achieved after the local heating protocol, each site was normalized to maximal vasodilation by perfusing each microdialysis site with 28 mM sodium nitroprusside (SNP; Nitropress, Abbott Laboratories, Chicago, IL) at a rate of 4 μl/min and locally heating the control site to 43°C. This dose of SNP and level of local heating have been shown previously to elicit maximal vasodilation in human skin (25, 29).

**Protocol 2: H2-receptor antagonist studies.** This protocol was designed as a parallel to protocol 1 to determine the contribution of H2-receptor activation to cutaneous thermal hyperemia to local heating. Six subjects (3 women and 3 men) participated in this protocol. Subjects were instrumented as in protocol 1 and as described above. The first microdialysis site was perfused with 2 mM of the H2-receptor antagonist cimetidine (Sigma). Our laboratory has previously shown that this dose of cimetidine in human skin significantly attenuates the vasodilation to exogenous histamine (40). The second microdialysis site was perfused with 10 mM l-NAME to inhibit NO synthase. The third site was perfused with cimetidine and l-NAME (2 and 10 mM final concentrations, respectively) and was used to determine any potential interaction between H2-receptor activation and NO during local skin heating. A fourth site without a microdialysis fiber served as a control site.

As in protocol 1, all drugs were perfused at a rate of 2 μl/min for 30–45 min before the local heating protocol and were continuous for the duration of the local heating. After the 30-min drug infusion, the local heaters were increased from 33 to 42°C as described above.
Once a plateau in skin blood flow was achieved, each microdialysis site was normalized to maximal vasodilation via 28 mM SNP infusion and the control site was normalized to maximal vasodilation via local heating to 43°C.

**Data collection and statistical analysis.** Data were digitized and stored at 20 Hz on a personal computer. Data were analyzed offline using signal processing software (Windaq, Data Instruments, Akron, OH). Cutaneous vascular conductance (CVC) was calculated as RBC flux (mV) \( \div \) mean arterial pressure (mmHg) and is expressed as a percentage of maximal vasodilation (%CVCmax) via SNP infusion or local heating to 43°C.

Because of the transient nature of the initial peak and nadir response, a stable 30- to 60-s period of skin blood flow was used for analysis. For the plateau during local heating and maximal skin blood flow, a stable 5- to 10-min period of skin blood flow was used for subsequent analysis.

The time to onset of the initial peak was taken as the time to reach the highest point after the initiation of the local heating protocol. Time to onset of the nadir response was taken as the time to reach the lowest point after the initiation of the heating protocol. The time required to reach a stable plateau in skin blood flow after the initiation of the heating protocol was taken as the time to onset of the secondary plateau phase.

For each protocol, a one-way ANOVA with repeated measures was used to compare the initial peak, nadir, and secondary plateau responses in all four sites to determine the relative contribution of histamine-receptor activation and NO as well as the interaction between histamine-receptor activation and NO. Similarly, a one-way ANOVA with repeated measures was used to analyze the time to onset of the initial peak, nadir, and secondary plateau in each protocol. The Holm-Sidak post hoc test was used to determine where significance occurred. A \( P \) value < 0.05 was considered statistically significant, and all data are presented as means \( \pm \) SE.

**RESULTS**

Figure 1 is a representative skin blood flow tracing of a control site and an H1-receptor antagonist site from one subject.

**Protocol 1: H1-receptor antagonist studies.** The group data for the initial peak response in all four sites are summarized in Fig. 2A. There was no statistical difference between initial peak CVC in control (77 \( \pm \) 4%CVCmax) and pyrilamine (74 \( \pm \) 6%CVCmax) sites. Similarly, there was no statistical difference between initial peak CVC in L-NAME (47 \( \pm \) 7%CVCmax) and pyrilamine plus L-NAME (49 \( \pm \) 6%CVCmax) sites. However, L-NAME and pyrilamine plus L-NAME sites were significantly reduced compared with both control and pyrilamine only sites (\( P < 0.01 \) for all conditions). The time to initial peak averaged 4.1 \( \pm \) 0.3, 3.6 \( \pm \) 0.2, 3.7 \( \pm \) 0.2, and 3.9 \( \pm \) 0.2 min in control, pyrilamine, L-NAME, and combined pyrilamine plus L-NAME sites, respectively. There were no statistical differences between sites in time to onset of the initial peak.

The nadir in control sites averaged 60 \( \pm \) 8%CVCmax. The nadir in pyrilamine (39 \( \pm \) 6%CVCmax), L-NAME (15 \( \pm \) 3%CVCmax), and pyrilamine plus L-NAME (15 \( \pm \) 2%CVCmax) sites were all significantly reduced compared with the nadir in control sites (\( P < 0.01 \) for all conditions). The nadir in L-NAME and pyrilamine plus L-NAME sites was significantly reduced compared with the pyrilamine only sites (\( P < 0.01 \)). However, there was no statistical difference between L-NAME and pyrilamine plus L-NAME sites. These data are summarized in Fig. 2B. The time to nadir averaged 6.7 \( \pm \) 0.4, 7.1 \( \pm \) 0.3, 7.2 \( \pm \) 0.4, and 7.0 \( \pm \) 0.6 min in control, pyrilamine, L-NAME, and combined pyrilamine plus L-NAME sites, respectively.
There were no statistical differences between sites in time to onset of the nadir.

Figure 2C summarizes the group data for the secondary plateau response to local skin heating. Secondary plateau values averaged 84 ± 4, 84 ± 5, 39 ± 8, and 39 ± 9% CVC\textsubscript{max} in control, pyrilamine, l-NAME, and pyrilamine plus l-NAME sites, respectively. There was no statistical difference between control and pyrilamine sites or between l-NAME and pyrilamine plus l-NAME sites. However, the l-NAME and the pyrilamine plus l-NAME sites were significantly reduced compared with control (P < 0.001) and pyrilamine sites (P < 0.001). The time to onset of the secondary plateau averaged 35 ± 5 min in control sites, 36 ± 3 min in pyrilamine sites, 39 ± 3 min in l-NAME sites, and 40 ± 3 min in combined pyrilamine plus l-NAME sites. The time to onset of the secondary plateau was not statistically different between sites.

Protocol 2: H\textsubscript{2} antagonist studies. There was no statistical difference in initial peak CVC between control (70 ± 6% CVC\textsubscript{max}) and cimetidine (62 ± 6% CVC\textsubscript{max}) sites. Similarly, l-NAME (48 ± 7% CVC\textsubscript{max}) and cimetidine plus l-NAME (45 ± 3% CVC\textsubscript{max}) sites were not statistically different from each other. However, the l-NAME and cimetidine plus l-NAME sites were significantly attenuated compared with both control and cimetidine only sites (P < 0.01 for all conditions). The group data are summarized in Fig. 3A. The time to onset of the initial peak averaged 4.2 ± 0.3 min in control sites, 4.1 ± 0.2 min in cimetidine sites, 4.1 ± 0.3 min in l-NAME sites, and 3.8 ± 0.2 min in combined cimetidine plus l-NAME sites. The time to onset of the initial peak was not statistically different between sites.

The nadir in control (63 ± 6% CVC\textsubscript{max}) and cimetidine (57 ± 6% CVC\textsubscript{max}) were not statistically different (P = 0.220), and there was no statistical difference (P = 0.290) between l-NAME (15 ± 4% CVC\textsubscript{max}) and cimetidine plus l-NAME (11 ± 2% CVC\textsubscript{max}) sites. Compared with control and cimetidine sites, the nadir in l-NAME and cimetidine plus l-NAME sites was significantly attenuated (P < 0.001 for all conditions). These data are shown in Fig. 3B. The time to onset of the nadir averaged 6.8 ± 0.5 min in control sites, 6.7 ± 0.4 min in cimetidine sites, 7.1 ± 0.4 min in l-NAME sites, and 7.0 ± 0.3 min in combined cimetidine plus l-NAME sites. There was no statistical difference between sites.

The group data for the secondary plateau response are shown in Fig. 3C. The secondary plateau averaged 86 ± 4, 88 ± 5, 42 ± 8, and 31 ± 6% CVC\textsubscript{max} in the control, cimetidine, l-NAME, and cimetidine plus l-NAME sites, respectively. There was no statistical difference between the control and cimetidine sites or between the l-NAME and cimetidine plus l-NAME sites. Compared with control and cimetidine sites, the l-NAME and cimetidine plus l-NAME sites were significantly attenuated (P < 0.001 for all conditions). The time to onset of the secondary plateau averaged 36 ± 3 min in control sites, 34 ± 2 min in cimetidine sites, 36 ± 3 min in l-NAME sites, and 38 ± 4 min in combined cimetidine plus l-NAME sites. The time to onset of the secondary plateau was not statistically different between sites.

**DISCUSSION**

In this study, we found that H\textsubscript{1}-receptor activation does not contribute to the initial peak or secondary plateau phases of the thermal hyperemic response to local heating of the skin. However, H\textsubscript{1}-receptor activation contributes modestly to the nadir response (Fig. 2B). These data also suggest that there is a modest interaction between NO and H\textsubscript{1} receptors in the nadir response. That is, a portion of the NO component of the nadir response can be explained by H\textsubscript{1}-receptor activation. In contrast, the H\textsubscript{2} isoform of the histamine receptor does not appear to contribute to any phase of thermal hyperemia to local skin heating, and there does not appear to be an interaction between NO and H\textsubscript{2} receptors (Fig. 3, A–C).

The use of local skin heating and laser-Doppler flowmetry is commonly used as a noninvasive clinical tool to assess microvascular and endothelial function in various patient groups (1, 4, 35, 36, 41). Postocclusive reactive hyperemia has been used as a noninvasive clinical tool to assess microvascular function for many years. However, our laboratory has shown that reactive hyperemia in the cutaneous circulation does not contain an NO component, thus limiting its utility as a noninvasive test of endothelial function (42). Because the thermal hyperemic response to local skin heating contains a substantial NO component (25, 29), local heating may serve as a useful clinical tool to assess endothelial function. It is therefore important to understand the mechanisms of the thermal hyperemic response to local skin heating in healthy control subjects.

The reason why the H\textsubscript{2} antagonist attenuated the nadir response but was without effect on the initial peak and secondary plateau is unclear. It has been suggested that substance P and CGRP are coreleased from C-fiber afferent nerves in response to direct local heating of the skin (28, 29). These neuropeptides have been shown to be colocalized in nerve
H1-receptor activation may be sufficient to minimize the dephases. However, the increase in histamine and subsequent flow, such as during the initial peak or secondary plateau that would contribute substantially to the increased skin blood response to H1-receptor activation has been shown to work and/or the secondary plateau response, because vasodilation in skin blood flow during the nadir response. Additionally, both peptides have been shown to induce NO release from dermal endothelial cells (9), and vasodilation in both peptides has been shown to be attenuated in the presence of an NO synthase inhibitor (16, 26, 44), and intradermal histamine has been shown to increase NO concentration in human skin (11, 12). Thus, if substance P and CGRP are indeed involved in the thermal hyperemic response to local heating of the skin, they may partially exert their vasodilatory effects through NO and through direct actions on the vasculature. However, to date there have been no studies providing direct evidence for either peptide in the cutaneous response to direct local heating.

A possible scenario is substance P is released during thermal hyperemia to local skin heating and causes mast cell degranulation, but the concentration of histamine does not reach vasoactive levels. This would be in line with data from Petersen et al. (32), who reported that at least 100 nM concentration of histamine is required to appreciably increase skin blood flow. Furthermore, Barnes and colleagues (2) reported that histamine does not act as the final vasodilator in substance P-induced axon reflexes. In the context of data reported in the present study, it is possible there is an increase in histamine concentration during local heating and that the level of increase is sufficient to activate some H1 receptors, but not to a level that would contribute substantially to the increased skin blood flow, such as during the initial peak or secondary plateau phases. However, the increase in histamine and subsequent H1-receptor activation may be sufficient to minimize the decrease in skin blood flow during the nadir response.

If the above scenario is true, it raises the question of why we failed to observe even a modest attenuation of the initial peak and/or the secondary plateau response, because vasodilation in response to H1-receptor activation has been shown to work through the production of local vasodilators such as NO (8, 21). There are at least two possibilities to address this. First, it may be that one or more locally produced vasodilators, other than NO, are involved in the thermal hyperemic response to local skin heating. Possible candidates are adenosine and prostaglandins, which have been shown to be involved in axon reflex-mediated vasodilation in human skin (3, 13) and have been shown to be produced in response to H1-receptor activation (8, 21). Golay and colleagues (15) recently found no evidence of a prostanoid-mediated component to the thermal hyperemic response to local skin heating. However, a role for vasoactive prostanoids cannot be completely ruled out because the vasodilator pathways involved in thermal hyperemia to local heating are redundant and involve a complex series of interactions. As such, inhibition of the cyclooxygenase pathway may not reveal a role for vasoactive prostanoids in thermal hyperemia to local heating, but it may be unmasked when combined with an NO synthase inhibitor or when another vasodilator pathway is blocked. Second, if the neuropeptides substance P and CGRP are indeed involved in thermal hyperemia to local heating, it may be that these peptides 1) induce vasodilation via direct production of NO or 2) directly stimulate their respective receptors to elicit smooth muscle relaxation. Clearly, more research is needed to fully elucidate mechanisms underlying the cutaneous response to direct local heating.

Although we did not observe an effect of an H2-receptor antagonist on the thermal hyperemic response to local heating of the skin, these data are consistent with previous reports from our laboratory in which we found no effect of an H2-receptor antagonist to exogenous VIP-mediated vasodilation or during whole body heat stress (40, 43). However, we are confident that the lack of an effect cannot be attributed to the properties of the H2 antagonist used in this study as we have previously shown that 2 mM cimetidine significantly attenuates the vasodilation to exogenous histamine (40). From these data, it appears that a substance released in response to exogenous VIP and under conditions of whole body heating and local heating of the skin is specific for the H1 isoform of the histamine receptor.

In conclusion, the data from this study suggest that there is a modest role for H1-receptor activation in the nadir response, but not the initial peak or secondary plateau phase, to local heating of human skin. The data from the H1-receptor antagonist studies also suggest that a portion of the NO component of the nadir response can be explained by H1-receptor activation. Furthermore, we have provided evidence that H2-receptor activation does not significantly contribute to any phase of the local heating response in the skin.

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