Prostanoids contribute to cutaneous active vasodilation in humans

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Prostanoids contribute to cutaneous active vasodilation in humans. Am J Physiol Regul Integr Comp Physiol 291: R596–R602, 2006. First published February 16, 2006; doi:10.1152/ajpregu.00710.2005.—The specific mechanisms by which skin blood flow increases in response to a rise in core body temperature via cutaneous active vasodilation are poorly understood. The primary purpose of this study was to determine whether the cyclooxygenase (COX) pathway contributes to active vasodilation during whole body heat stress (protocol 1; n = 9). A secondary goal was to verify that the COX pathway does not contribute to the cutaneous hyperemic response during local heating (protocol 2; n = 4). For both protocols, four microdialysis fibers were placed in forearm skin. Sites were randomly assigned and perfused with 1) Ringer solution (control site); 2) ketorolac (KETO), a COX-1/COX-2 pathway inhibitor; 3) N9-nitro-l-arginine methyl ester (l-NAME), a nitric oxide synthase inhibitor; and 4) a combination of KETO and l-NAME. During the first protocol, active vasodilation was induced using whole body heating with water-perfused suits. The second protocol used local heaters to induce a local hyperemic response. Red blood cell flux (RBC flux) was indexed at all sites using laser-Doppler flowmetry, and cutaneous vascular conductance (CVC; RBC flux/mean arterial pressure) was normalized to maximal vasodilation at each site. During whole body heating, CVC values at sites perfused with KETO (43 ± 9% CVCmax), l-NAME (35 ± 9% CVCmax), and combined KETO/l-NAME (22 ± 8% CVCmax) were significantly decreased with respect to the control site (59 ± 7% CVCmax) (P < 0.05). Additionally, CVC at the combined KETO/l-NAME site was significantly decreased compared with sites infused with KETO or l-NAME alone (P < 0.05). In the second protocol, the hyperemic response to local heating did not differ between the control site and KETO site or between the l-NAME and KETO+l-NAME site. These data suggest that prostanoids contribute to active vasodilation, but do not play a role during local thermal hyperemia.

local heating; skin; microdialysis; hyperthermia; nitric oxide

NONGLABROUS CUTANEOUS BLOOD flow is controlled by two branches of the sympathetic nervous system: the sympathetic noradrenergic branch, which is responsible for vasoconstriction and the sympathetic cholinergic branch, which is responsible for active vasodilation. Under thermoneutral resting conditions, cutaneous blood vessel tone is maintained by sympathetic noradrenergic release of norepinephrine that binds to α1- and α2-receptors. Consequently, when core body temperature is challenged during whole body heat stress, the initial 5–15% rise in skin blood flow is due to withdrawal of this tonic noradrenergic vasoconstriction. The remaining 85–95% increase in skin blood flow is mediated by sympathetic cholinergic active vasodilation.

Though the specific mechanisms responsible for active vasodilation have yet to be elucidated, many substances have been implicated as contributors to the resultant vasorelaxation. Previous work has suggested a role for nitric oxide (NO) (21, 33, 40), vasoactive intestinal peptide (VIP) (1), ACh (20, 26, 36), and H1 receptors (42). In addition, there appears to be some overlap in the vasodilatory pathways of these substances in the skin, particularly with respect to NO. In human skin, VIP has been shown to have both NO and H1-receptor components (39); ACh has been shown to be partially dependent on NO in some (23, 36), but not all, studies (17); and H1-receptor activation also appears to have a NO-dependent component (42).

When NO synthase (NOS) is inhibited by N9-nitro-l-arginine methyl ester (l-NAME) during whole body heating, active vasodilation is reduced by 30–40% (21, 33, 40). The main mechanism through which NO reduces vascular tone is via activation of soluble guanylate cyclase (11). Additionally, NO is also capable of upregulating cyclooxygenase type-1 (COX-1) and type-2 (COX-2) enzymes (7, 9, 10, 30–32, 38) in a variety of cell types. COX-1/COX-2 enzymes are rate-limiting enzymes that are a part of the prostaglandin H-synthase complex involved in the production of prostanoids from arachidonic acid in the endothelium and have been found in human skin (15).

In addition to NO, H1 receptors and ACh have also been linked to prostanoid production. For example, two recent investigations observed a diminished rise in CVC during ACh infusion when the COX-pathway was blocked (17, 23). H1 receptor activation has been demonstrated to increase the activity of phospholipase A2, the enzyme responsible for the production of arachidonic acid from phospholipid precursors (16, 19). Because there is evidence that NO, ACh, and H1 receptors modulate active vasodilation and the production of prostanoids, this raises the possibility that the COX pathway may be involved in active vasodilation.

Vasodilator mechanisms that underlie the cutaneous hyperemic response to rapid, nonpainful, local heating of human skin are different than those involved in cutaneous active vasodilation. Within minutes of direct application of heat, there is a rapid and transient increase in skin blood flow termed the initial peak, followed by a brief nadir response, and then followed by a more prolonged secondary rise in skin blood flow.
flow to a plateau (25). The initial peak is thought to be an axon reflex-mediated response, whereas the secondary plateau is mediated mainly by NO (22, 27, 28). In an investigation by Golay et al. (14), the hyperemic response to local heating was examined during COX-pathway blockade using oral intake of aspirin. Their findings suggested that the COX pathway does not play a role in the vasodilatory response to local heating. Our goal in the second protocol was to verify these findings using the microdialysis infusion of a COX-1/COX-2 inhibitor, as we and others have previously found that systemic or arterial infusions do not consistently block responses in the forearm skin (12, 34).

The goal of the current studies was threefold: 1) to determine whether prostanoids contribute to active vasodilation, 2) to determine whether there is an interaction between NO and COX pathways during active vasodilation, and 3) to verify that prostanoids are not involved in the vasodilatory response to local heating using the microdialysis technique. We therefore tested the hypotheses that COX inhibition would diminish cutaneous active vasodilation but would not alter the vasodilatory response to local heating.

METHODS

Subjects. A total of 13 subjects participated in one of two protocols. An additional two subjects participated in a follow-up protocol. Five males (mean age 22 ± 1 years) and four females (mean age 23 ± 1 years) participated in the first protocol, while four males (mean age 21 ± 1 years) participated in the second protocol. The follow-up protocol consisted of one female (age 26 years) and one male (age 21 years). The five females were taking oral contraceptives (OC) and were studied during menstruation as the phase of OC use is known to increase from a baseline of 33°C by 0.5°C every 5 s, up to 42°C at each of the four sites. This results in an increase in skin temperature to ~40°C (28). Subjects did not report any sensations of pain during these procedures. After placement of the fibers, drugs were infused for 75 min before baseline skin blood flow measurements and a brachial blood pressure were then taken. The temperatures of the local heaters were then increased to 43°C for 30 min in addition to the infusion of 28 mM SNP to elicit maximal vasodilation. The 10 mM KETO site and 10 mM KETO/NAME site each had the temperature of the local heaters increased to 43°C for 30 min in addition to the infusion of 28 mM SNP to elicit maximal vasodilation.

Protocol 2. This protocol was designed to test whether the increase in CVC in response to local heating would be altered by COX-inhibition. After four microdialysis fibers were placed, they were taped down and perfused with either lactated Ringer solution (control site), 10 mM KETO, 10 mM NAME, or a combination of 10 mM KETO/NAME. The four sites were instrumented for measurement of skin blood flow using laser Doppler flowmetry coupled with integrated local heaters. Drugs were infused for 75 min before baseline skin blood flow measurement to ensure adequate blockade (17) and also allow the insertion trauma to subside. Ten minutes of baseline skin blood flow measurements and a brachial blood pressure were then taken. The temperatures of the local heaters were then increased from a baseline of 33°C by 0.5°C every 5 s, up to 42°C at each of the four sites. This results in an increase in skin temperature to ~40°C (28). Subjects did not report any sensations of pain during these procedures. After placement of the fibers, drugs were infused for 75 min before baseline skin blood flow measurements and a brachial blood pressure were then taken. The temperatures of the local heaters were then increased to 43°C for 30 min in addition to the infusion of 28 mM SNP to elicit maximal vasodilation. The 10 mM KETO site and 10 mM KETO/NAME site each had the temperature of the local heaters increased to 43°C for 30 min in addition to the infusion of 28 mM SNP to elicit maximal vasodilation. Once maximal plateau had been reached, a brachial blood pressure was taken and the experiment was ended.

Follow-up protocol. This protocol was designed to substantiate findings from protocol 1 by using a separate COX inhibitor to verify the involvement of the COX pathway during the active vasodilatory response. Experimental setup was identical to that of protocol 1, with the exception of the use of 10 mM KETO. In the place of 10 mM KETO, 10 mM acetylsalicylic acid (ASA) was infused to inhibit the COX pathway. Acetylsalicylic acid, more commonly known as aspirin is a COX-1/COX-2 inhibitor. ASA was solvated with the use of Ringer solution. 15 min of gentle heat, and a magnetic stir bar. In addition to this modification, integrated local heaters were used at all four sites in addition to 28 mM SNP to ensure maximal vasodilation was achieved after the cessation of whole body heating.

Statistical analysis. Data were digitized and stored on a computer at 40 Hz. Data were analyzed off-line with signal processing software (WinDaq, Datqaq Instruments, Akron, OH). CVC was averaged over stable 20-s periods at each time point. Paired Student’s t-tests were used to determine significant differences between before whole body heating HR, MAP, and TΔm to those resulting from prolonged whole body heat stress. A repeated measures two-way ANOVA was used to detect differences in CVC between drug treatment sites and state of
whole body heating. The same test was also used to detect differences in CVC between drug treatment sites for time to 50% plateau (TP50%) and local heating data. For the two-way ANOVA, Tukey's post hoc analysis was used. Significance was set at the $\alpha = 0.05$ level, and values are displayed as means ± SE.

**RESULTS**

**Protocol 1.** Average HR increased significantly during whole body heat stress ($P < 0.05$) whereas MAP did not change. Core body temperature increased by an average of 1.00 ± 0.40°C above resting body temperature during whole body heating ($P < 0.05$) (Table 1).

Figure 1 displays a representative tracing of CVC throughout whole body heating at all four sites as a function of time. CVC was not significantly different during baseline measurements among the four microdialysis sites. During whole body heating, however, CVC values at sites perfused with 10 mM KETO (43 ± 9% CVC$_{\text{max}}$), 10 mM L-NAME (35 ± 9% CVC$_{\text{max}}$), and combined 10 mM KETO/L-NAME (22 ± 8% CVC$_{\text{max}}$) were significantly decreased with respect to the control site (59 ± 7% CVC$_{\text{max}}$) ($P < 0.05$). Additionally, CVC at the combined KETO/L-NAME site was significantly decreased compared with sites infused with KETO or L-NAME alone ($P < 0.05$). Although the 10 mM KETO site was slightly higher on average, there was no significant difference in CVC between the 10 mM KETO and 10 mM L-NAME sites as a result of whole body heating. Mean responses are displayed in Fig. 2.

To compare the time course of active vasodilation between experimental sites, TP50% was calculated by measuring the time from the onset of whole body heat stress to the time that RBC$_{\text{flux}}$ had reached 50% of plateau at each experimental site. The 50% plateau RBC$_{\text{flux}}$ value was calculated using the following equation $\left[\frac{(\text{RBC}_{\text{flux}} \text{ Plateau} - \text{RBC}_{\text{flux}} \text{Baseline})}{2} + \text{RBC}_{\text{flux}} \text{Baseline}\right]$. The 10 mM L-NAME (2,600 ± 969 s) and 10 mM KETO/L-NAME (2,500 ± 859 s) sites had significantly greater TP50% compared with control (1,933 ± 674 s) and 10 mM KETO (1,886 ± 661 s) sites ($P < 0.05$). There was no significant difference between control and 10 mM KETO sites.

Raw maximum CVC values, calculated as RBC$_{\text{flux}}$/MAP, were analyzed to detect differences in maximum CVC between sites that received 28 mM SNP and local heating to those that received 28 mM SNP alone. There was no statistically significant difference ($P > 0.05$); however, there was a tendency for sites infused with 10 mM KETO to have a lower raw maximum CVC on average: control site (3.6 ± 0.9 CVC), 10 mM KETO (2.9 ± 0.9 CVC), 10 mM L-NAME (3.5 ± 0.6 CVC), and 10 mM KETO/L-NAME (3.0 ± 0.7 CVC).

Table 1. Comparison of data between resting and plateau of skin blood flow during whole body heating

<table>
<thead>
<tr>
<th></th>
<th>Resting</th>
<th>Whole Body Heating</th>
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<tr>
<td>MAP, mmHg</td>
<td>88 ± 2</td>
<td>90 ± 3</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>65 ± 2</td>
<td>97 ± 3*</td>
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<tr>
<td>Tcore, °C</td>
<td>36.1 ± 0.1</td>
<td>37.1 ± 0.2*</td>
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Values are means ± SE; MAP, mean arterial pressure; HR, heart rate; Tcore, core body temperature. *$P < 0.05$; significant difference from resting to whole body heating.

**Protocol 2.** Average HR and MAP did not change throughout the protocol. Figure 3 displays a representative tracing of CVC throughout skin local heating up to a temperature of 42°C among experimental sites. Baseline CVC values did not differ between experimental sites. Group data for the initial peak response in all four sites are summarized in Fig. 4A. There was no statistical difference between initial peak CVC in control (74 ± 2% CVC$_{\text{max}}$) and KETO (70 ± 2% CVC$_{\text{max}}$). Similarly, there was no statistical difference between initial peak CVC in L-NAME (54 ± 2% CVC$_{\text{max}}$) and KETO/L-NAME (53 ± 4% CVC$_{\text{max}}$). However, L-NAME and KETO/L-NAME sites were significantly reduced with respect to both control and KETO sites ($P < 0.05$).

There was no statistical difference between nadir CVC response in control (56 ± 7% CVC$_{\text{max}}$) and KETO (50 ± 4% CVC$_{\text{max}}$) sites. Similarly, there was no statistical difference between nadir CVC response in L-NAME (20 ± 3% CVC$_{\text{max}}$) and KETO/L-NAME (21 ± 3% CVC$_{\text{max}}$) sites. There was a significant difference between the CVC values at KETO/L-NAME sites and baseline.

Fig. 1. Representative tracing of cutaneous vascular conductance (CVC) from baseline to plateau at the end of whole body heating at four microdialysis sites (n = 1). Solid circles, control site; solid triangles, treatment with 10 mM ketorolac (KETO); open circles, treatment with 10 mM N$^\text{G}$-nitro-L-arginine methyl ester (l-NAME); open triangles, treatment with 10 mM KETO/10 mM l-NAME.

Fig. 2. Bar graphs represent mean CVC values as a percentage of maximum ± SE during baseline and at four different skin sites (n = 9) as a result of whole body heating; Treatment with 10 mM L-NAME alone, 10 mM KETO alone, and 10 mM KETO + 10 mM L-NAME significantly decreased CVC compared with control site. *$P < 0.05$, significant vs. control site; #P < 0.05, significant vs. 10 mM KETO site and 10 mM l-NAME site.
and KETO/L-NAME (18 ± 7% CVCmax). However, L-NAME and KETO/L-NAME sites were significantly reduced compared with both control and KETO sites (P < 0.05). The group data for the nadir response in all four sites are summarized in Fig. 4B.

Figure 4C summarizes the secondary plateau response to local skin heating. There was no statistical difference between control (79 ± 6% CVCmax) and KETO (81 ± 4% CVCmax) sites or between L-NAME (41 ± 6% CVCmax) and KETO/L-NAME (41 ± 7% CVCmax) sites. However, L-NAME and KETO/L-NAME sites were significantly reduced compared with control and KETO sites (P < 0.05).

**Follow-up protocol.** Average HR increased during whole body heat stress in both subjects, while MAP did not change. Core body temperature increased 0.8°C in the first subject, and 0.7°C in the second subject above resting body temperature during whole body heating.

CVC was not different during baseline measurements among the four microdialysis sites. During whole body heating, however, CVC values at sites perfused with 10 mM ASA (48% CVCmax, and 25% CVCmax), 10 mM L-NAME (44% CVCmax and 35% CVCmax), and combined 10 mM ASA/L-NAME (28% CVCmax and 15% CVCmax) were decreased with respect to the control site (82% CVCmax and 44% CVCmax). Additionally, CVC at the combined ASA/L-NAME site was decreased compared with sites infused with ASA or L-NAME alone.

**DISCUSSION**

The primary purpose of this study was to determine whether the COX pathway contributes to active vasodilation. When the COX pathways were blocked, CVC was significantly decreased during whole body heating. Furthermore, the simultaneous blockade of NOS and COX pathways unmasked an even greater decrease in CVC than observed with NOS or COX-pathway inhibition alone, suggesting that NO and the COX-pathways contribute to a portion of active vasodilation independent of one another. During the second protocol, COX-pathway inhibition did not affect the skin blood flow response to local heating, thereby confirming a previous report in which the COX pathways were inhibited using oral ibuprofen (14).

These findings, paired with the observation of similar reductions in active vasodilation with the use of acetylsalicylic acid, suggest the reduction in CVC observed during whole body heating was the result of COX inhibition and not simply a nonspecific effect of ketorolac on the ability of smooth muscle to relax. Consistent with our hypothesis, these data suggest that the COX pathways contribute to cutaneous active vasodilation but do not contribute to the hyperemic response to local heating.

In a previous study by Charkoudian et al. (5), COX-pathway inhibition with the use of oral ibuprofen did not appear to alter the magnitude or onset of active vasodilation. Data from the present study are consistent with these findings with respect to the effects of COX inhibition on the onset of active vasodila-
tion; however, our findings differ in that we found COX inhibition to alter the magnitude of the active vasodilatory response. The diminished active vasodilatory response observed during COX inhibition in the present study is most likely explained by methodological differences between our study and that of Charkoudian et al. (5). In the study by Charkoudian et al., the goal of the experiment was to observe whether the onset of active vasodilation was altered by COX inhibition during different hormonal phases in females. Thus subjects were only heated to between 0.2 and 0.4°C above thermoneutral core body temperature and were not exposed to prolonged heat stress. In contrast, the core body temperature of subjects in the present study were increased to a much greater extent, as the goal of this investigation was to observe the effects of COX inhibition on the capacity of the active vasodilatory response.

Prostanoid production during active vasodilation could be elicited by a variety of mechanisms. One such mechanism could be the release of an unknown neurotransmitter and/or vasoactive substance that vasodilates, at least in part, through increasing the production of vasodilatory prostanoids. Endothelial H1-receptors activation have three primary effects, 1) increasing endothelial intracellular Ca2+, 2) increasing endothelial NO, and 3) upregulating phospholipase A2, the enzyme responsible for producing arachidonic acid (16, 19). Thus vasodilation attributable to the COX pathway could simply be a function of H1-receptor activation during active vasodilation.

In a previous study from our laboratory, when the H1-receptor antagonist pyrilimine was administered during whole body heating, CVC was partially attenuated, suggesting H1-receptor involvement in active vasodilation (42). However, when NOS was inhibited in combination with H1-receptors, the drop in CVC was similar to NOS inhibition alone, suggesting that H1 receptor-mediated vasodilation works primarily through NO. Because, as argued below, the NO and COX pathway-dependent components appear to be independent in the present study, the present findings suggest that H1-receptor activation was not responsible for increased prostanoid production.

ACh released during whole body heat stress may also contribute to active vasodilation. Using atropine iontophoresis, Kellogg et al. (20) blocked muscarinic receptors and observed that the rise in CVC was attenuated by almost 30% during whole body heat stress before reaching a stable plateau (20). In contrast, Shastry et al. (35) administered atropine after CVC had reached a plateau during whole body heat stress and observed no change in CVC, thus providing evidence that ACh does not contribute to the later phase of active vasodilation. In another study by Shibisaki et al. (36), investigating ACh during whole body heating, inhibition of acetylcholinesterases with neostigmine produced a leftward shift in the onset of active vasodilation. This leftward shift was then offset by coadministration of a NOS inhibitor, suggesting that ACh works primarily through NO-dependent mechanisms in contributing to the initial rise in skin blood flow during active vasodilation. Along these lines, Kellogg et al. (23) recently provided evidence that ACh-mediated vasodilation was dependent on NO and prostanoids. Results from their investigation showed that inhibition of the NOS and COX pathways produced a greater attenuation of CVC than that of NOS or COX inhibition alone. Holowatz et al. (17) also observed that the COX pathway contributed to ACh-mediated vasodilation; however, in contrast to Kellogg et al. (23), they did not observe a role for NO (17). The difference in results may be accounted for by differences in methodology, as Holowatz et al. (17) used a 137.5-μM bolus dose of ACh, whereas Kellogg et al. (23) looked at the prolonged effects of a continuous 16-mM infusion of ACh. Because both studies indicated a clear role for the COX pathway, and thus vasodilatory prostanoid production during ACh-mediated vasodilation, there is a possibility that vasodilation attributable to ACh during active vasodilation could be working through increasing the production of vasodilatory prostanoids.

It has been demonstrated that NO contributes significantly to active vasodilation (21, 33), and the vasodilation induced by a number of possible substances involved in active vasodilation (36, 39, 41, 42). Along these lines, NO has also been observed to increase the biosynthesis of prostanoids in numerous experimental models. In two different studies by Salvemini et al. (31, 32), the introduction of the NOS donor, SNP, increased prostaglandin production by up to 50%. In the same experimental model, NOS inhibitors resulted in decreased prostaglandin production (30–32). These findings suggest the possibility that NO released during active vasodilation may increase the production of prostanoids. Contrary to these findings, however, others have observed prostaglandin biosynthesis to be stunted in the presence of NO (25), and one study even found prostaglandin biosynthesis to attenuate NO production (37). These contradictory findings may be explained by the difference in cell types used for experimental procedures along with variation within blocking agents being used. In the current study, the combined blockade of NOS and COX pathways resulted in a reduction in CVC that was almost equal to the reduction in CVC at the individually blocked sites added together. These data could suggest that the COX pathway and NO work independently of one another during active vasodilation.

Another possibility is that COX-pathway upregulation could be flow mediated. Several studies have established a role for the COX pathway during the postocclusion hyperemia response in the forearm (4, 13, 24, 29). However, conflicting results have been produced with regard to the investigation of this phenomenon in the cutaneous circulation. Dalle-Âve et al. (8) found that COX inhibitors infused intravenously did not alter the rise in skin blood flow produced by postocclusion hyperemia. Contrary to these data, two other groups observed diminished cutaneous reactive hyperemia after the administration of similar COX inhibitors (2, 3). It is, therefore, not possible at this time to rule out the possibility that the COX pathway is stimulated during active vasodilation through a shear stress mechanism.

In blocking the COX pathways during active vasodilation, the production of arachidonic acid, the prostaglandin precursor molecule, is not inhibited. Therefore, when COX enzymes are blocked by ketorolac during active vasodilation, arachidonic acid may be shuttled through a separate enzymatic pathway. Holowatz et al. (17) proposed that the production of arachidonic acid in the face of COX-inhibition in the skin could activate endothelial derived hyperpolarizing factors (EDHF).

They proposed that EDHFs could be responsible for a significant portion of ACh-mediated vasodilation because COX
inhibition only partially attenuated the response. Thus, with ACh infusion and active vasodilation, it is possible that the production of arachidonic acid during COX inhibition may result in the upregulation of EDHFs, thereby masking a more pronounced decrease in CVC. However, a role for EDHFs in these responses has not yet been investigated.

These data suggest that there is an increased production of vasodilatory prostanooids during active vasodilation. If the COX pathway is involved during whole body heating, cutaneous production of prostanooids would be expected to increase as a result. A limitation of these findings was that prostanooids were not assayed during whole body heating. Despite this limitation, however, the fact that COX pathway inhibition significantly decreased CVC during active vasodilation provides strong evidence in favor of the hypothesis presented.

A potential limitation to the current study was the inability to eliminate the possibility that perfusion of ketorolac disrupted blood vessel function independent of COX inhibition, thereby preventing full expression of vasodilation to whole body heating. One potential mechanism by which this could be occurring is that ketorolac may be upregulating the activity of the adrenergic vasconstrictor system and thus causing a superimposed vasoconstriction at sites perfused with ketorolac. This possibility seems fairly unlikely, however, as infusion of ketorolac during baseline conditions did not induce measurable vasoconstriction. In addition, we were unable to find any evidence to suggest ketorolac is capable of eliciting this type of response in the literature. Furthermore, the fact that a similar vasodilatory response to whole body heat stress was observed when ASA was used would suggest that the attenuated active vasodilatory response observed with the use of ketorolac was not due to a nonspecific secondary effect of the drug. In addition, our finding that COX-pathway inhibition did not affect the hyperemic response to local heating suggests that ketorolac did not disrupt the ability of blood vessels to vasodilate via neural (initial peak) or NO (secondary plateau) mechanisms. These findings do not eliminate the possibility that ketorolac may have interfered with other vasodilator pathways involved in active vasodilation. To minimize this likelihood, the drugs used for the investigation were carefully researched and dosages were assigned on the basis of prior investigations. A dose of 10 mM l-NAME has been used in numerous studies and has been shown to effectively inhibit the NOS production of NO (17, 21, 42). Similarly, 10 mM ketorolac has been shown to be sufficient to block the COX pathways (17). That is, significantly greater concentrations of the drugs do not result in a greater reduction in blood flow. Despite these efforts, it is possible that the antagonists used did not completely block the NOS and COX pathways. It is, therefore, not possible for us to eliminate the possibility that NO and or prostaglandins may play a larger role during active vasodilation than observed in the present investigation.

In conclusion, these data provide evidence supporting a role for the COX pathway during active vasodilation. Additionally, the COX-pathway appears to work independently of NO during active vasodilation and does not alter the hyperemic response to local heating. Together, these findings suggest vasodilatory prostanooid production increases during active vasodilation and thereby contributes to the rise in skin blood flow during whole body heat stress.

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