Ketorolac alters blood flow during normothermia but not during hyperthermia in middle-aged human skin

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Submitted 13 July 2009; accepted in final form 6 August 2009

Holowatz LA, Jennings JD, Lang JA, Kenney WL. Ketorolac alters blood flow during normothermia but not during hyperthermia in middle-aged human skin. J Appl Physiol 107: 1121–1127, 2009. First published August 6, 2009; doi:10.1152/japplphysiol.00750.2009.—In young healthy humans full expression of reflex cutaneous vasodilation is dependent on cyclooxygenase (COX)- and nitric oxide synthase (NOS)-dependent mechanisms. Chronic low-dose aspirin therapy attenuates reflex cutaneous vasodilation potentially through both platelet- and vascular COX-dependent mechanisms. We hypothesized the contribution of COX-dependent vasodilators to reflex cutaneous vasodilation during localized acute COX inhibition would be attenuated in healthy middle-aged humans due to a shift toward COX-dependent vasoconstrictors. Four microdialysis fibers were placed in forearm skin of 13 middle-aged (53 ± 2 yr) normotensive healthy humans, serving as control (Ringer), COX-inhibited (10 mM ketorolac), NOS-inhibited (10 mM Nω-nitro-L-arginine methyl ester), and combined NOS- and COX-inhibited sites. Red blood cell flux was measured over each site by laser-Doppler flowmetry as reflex vasodilation was induced by increasing oral temperature (T_or) 1°C using a water-perfused suit. Cutaneous vascular conductance was calculated (CVC = flux/mean arterial pressure) and normalized to maximal CVC (CVC_max; 28 mM sodium nitroprusside). CVC_max was not affected by localized microdialysis drug treatment (P > 0.05). Localized COX inhibition increased baseline (18 ± 3% CVC_max; P < 0.001) compared with control (9 ± 1% CVC_max), NOS-inhibited (7 ± 1% CVC_max), and combined sites (10 ± 1% CVC_max). %CVC_max in the COX-inhibited site remained greater than the control site with ΔT_or ≤ 0.3°C; however, there was no difference between these sites with ΔT_or ≥ 0.4°C. NOS inhibition and combined COX and NOS inhibition attenuated reflex vasodilation compared with control (P < 0.001), but there was no difference between these sites. Localized COX inhibition with ketorolac significantly augments baseline CVC but does not alter the subsequent skin blood flow response to hyperthermia, suggesting a limited role for COX-derived vasodilator prostanooids in reflex cutaneous vasodilation and a shift toward COX-derived vasoconstrictors in middle-aged human skin.

Skin blood flow; thermoregulation; prostaglandins; cyclooxygenase; nitric oxide

With rising body core temperature, skin blood flow is first increased by withdrawal of adrenergic vasoconstrictor tone, and then, on reaching a specific core temperature threshold, active cutaneous vasodilation occurs (25). Reflex vasodilation is mediated by cholinergic cotransmission (11) where several putative vasodilator mechanisms are involved, including the cotransmitter vasoactive intestinal peptide (1), histamine receptor activation (35), and neurokinin 1 receptor activation (34). Further, full expression of reflex cutaneous vasodilation in young healthy human skin is dependent on nitric oxide (NO) synthase (NOS) (10, 27) and cyclooxygenase (COX) second messenger mechanisms, each of which purportedly contributes independently to the rise in skin blood flow during hyperthermia (16).

With healthy human aging there is a significant attenuation in reflex cutaneous vasodilation due to a reduction in both NO- and cotransmitter-dependent vasodilation (5, 12). We have recently demonstrated that chronic low-dose aspirin therapy (81 mg) consistently and significantly attenuates reflex cutaneous vasodilation in middle-aged subjects (57 ± 3 yr) (6). Low-dose aspirin acetylates platelet COX-1 in the presystemic (portal) circulation (24), inhibiting COX for the life of the platelet (~10 days), whereas COX in vascular endothelial cells maintains the ability to produce COX-dependent vasodilators. However, the possibility exists that low-dose aspirin 1 inhibits cutaneous microvascular COX-1 (13, 32), thus inhibiting a key vascular enzyme involved in reflex vasodilation; and/or 2 inhibits platelets from directly releasing vasodilating factors such as NO, ATP, or 5-HT (3, 9, 20). In endothelial cells, COX synthesizes several vasodilator prostaglandins (PGI2, PGE, PGF, PGD), as well as vasoconstrictor (thromboxane) factors. With vascular aging there is a shift toward the latter, resulting in a proconstrictor state (31). For example, indirect evidence using exogenous acetylcholine-induced vasodilation in aged cutaneous vasculature suggests that there is a reduction in the synthesis of COX-derived vasodilator prostanooids and a shift toward COX-derived vasconstricting factors (7). However, the potential role for platelet vs. vascular COX and the effect of primary human aging on COX-dependent second messenger vasodilator and vasoconstrictor systems during hyperthermia are unclear.

The purpose of this study was to determine the effect of acute localized COX inhibition on reflex cutaneous vasodilation in healthy middle-aged human skin. We hypothesized that 1) localized nonspecific COX inhibition with ketorolac would increase baseline skin blood flow in a thermoneutral setting, and 2) the contribution of COX-derived vasodilator prostanooids to reflex cutaneous vasodilation would be attenuated in middle-aged humans.

METHODS

Subjects. Experimental protocols were approved by the Institutional Review Board at The Pennsylvania State University and conformed to the guidelines set forth by the Declaration of Helsinki. Verbal and written consent were voluntarily obtained from all subjects before participation. Studies were performed on 13 healthy subjects (Table 1).

Subjects underwent a complete medical screening, including a physician-supervised graded exercise test to evaluate the existence of underlying cardiovascular disease, blood chemistry, lipid profile evalu-

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Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Value</th>
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<tbody>
<tr>
<td>Sex, M/F</td>
</tr>
<tr>
<td>Age, yr</td>
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<tr>
<td>BMI, kg/m²</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
</tr>
<tr>
<td>HDL, mg/dl</td>
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<tr>
<td>LDL, mg/dl</td>
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<tr>
<td>Fasting blood glucose, mg/dl</td>
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<tr>
<td>SBP, mmHg</td>
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<tr>
<td>DBP, mmHg</td>
</tr>
<tr>
<td>MAP, mmHg</td>
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<tr>
<td>Baseline Tor, °C</td>
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Values are means ± SE; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP mean arterial pressure; Tor, oral temperature.

Microdialysis sites were perfused continuously for at least 75 min before the start of the baseline and during the baseline and heating periods with assigned pharmacological agents at a rate of 2.0 μl/min. Data were collected for 20 min before the start of whole body heating to ensure a true baseline value, after which whole body heating was initiated. After Tor had increased by 1°C from baseline and skin blood flow had reached an established plateau, mean body temperature was clamped by decreasing the temperature of the circulating water to 45°C. L-NAME (10 mM) was the perfused through the control site and the ketorolac site at 4.0 μl/min to quantify NO-dependent vasodilation. After laser-Doppler flux reached steady post-l-NAME values (~30–40 min), 28.0 mM sodium nitroprusside (Nitopress, Abbot Laboratories, Chicago, IL) was perfused through all sites at a rate of 4 μl/min to achieve maximal CVC (CVCmax) in combination with local heating of the skin to 43°C over each microdialysis site to ensure CVCmax had been achieved (16).

**Data acquisition and analysis.** Data were acquired using Windaq software and Dataq data-acquisition systems (Akron, OH). The data were collected at 40 Hz, digitized, recorded, and stored on a personal computer for further analysis. CVC data were averaged over 3-min periods for baseline and every 0.1°C rise in Tor and are presented as a percentage of CVCmax (%CVCmax). Thresholds for reflex vasodilation were determined by an experienced reviewer who was blinded to the microdialysis treatment sites as the point when laser-Doppler flux significantly deviated from baseline. Absolute CVCmax in each microdialysis site was calculated as the average of the stable plateau in laser-Doppler flux during 28 mM sodium nitroprusside infusion and local heating to 43°C divided by mean arterial pressure.

**Statistical analyses.** Initially, a three-way mixed models ANOVA was conducted to determine if there was a difference between the sexes on localized microdialysis drug treatments across the rise in oral core temperature. There was no difference between the sexes (P = 0.66) on the %CVCmax in microdialysis drug treatment sites across the rise in oral core temperature. Thereafter the sexes were combined and a two-way mixed models ANOVA with repeated measures was conducted to determine differences between localized microdialysis drug treatments across the rise in oral core temperature. Specific planned comparisons with Bonferroni corrections were performed when appropriate. Additionally, one-way ANOVA with repeated measures was conducted to determine differences in %CVCmax across the rise in oral core temperature. Specific planned comparisons with Bonferroni corrections were performed when appropriate. Additionally, one-way ANOVA with repeated measures was conducted to determine differences in %CVCmax across the rise in oral core temperature. Specific planned comparisons with Bonferroni corrections were performed when appropriate. Additionally, one-way ANOVA with repeated measures was conducted to determine differences in %CVCmax across the rise in oral core temperature. Specific planned comparisons with Bonferroni corrections were performed when appropriate.
due to drug treatments. The level of significance was set at $\alpha = 0.05$. Values are presented as means $\pm$ SE.

RESULTS

Subject characteristics are presented in Table 1. Absolute $T_{or}$ and $\Delta T_{or}$ thresholds for the onset of reflex cutaneous vasodilation are presented in Table 2. There was a rightward shift in the threshold for reflex vasodilation in sites treated with L-NAME and combined L-NAME + ketorolac ($P < 0.001$) for both the absolute $T_{or}$ threshold and $\Delta T_{or}$ compared with the control site. Localized ketorolac treatment alone did not change the threshold for reflex vasodilation compared with the control site ($P = 0.23$).

Group mean %CVCmax responses at (thermoneutral) baseline and with a 1.0°C rise in oral temperature are presented in Fig. 1, A and B, respectively. Localized COX inhibition with ketorolac increased thermoneutral baseline %CVCmax compared with all other sites ($P < 0.001$, Fig. 1A). L-NAME and ketorolac + L-NAME treatments decreased %CVCmax compared with control and ketorolac-treated sites ($P < 0.001$, Fig. 1B) during whole body heating. There was no difference in skin blood flow during hyperthermia in sites treated with ketorolac compared with control ($P = 1.0$).

Group mean %CVCmax responses across the rise in oral temperature are presented in Fig. 2. COX inhibition with ketorolac (Fig. 2A) increased %CVCmax compared with the control site at $\Delta T_{or} \geq 0.3°C$; however, there was no difference between these sites at $\Delta T_{or} \geq 0.4°C$. NOS inhibition with L-NAME at $\Delta T_{or} = 1.0°C$ resulted in a similar decline in %CVCmax in the ketorolac-treated site compared with control. L-NAME treatment (Fig. 2B) attenuated %CVCmax at $\Delta T_{or} \geq 0.3°C$ compared with the control site. In the control site %CVCmax after NOS inhibition with L-NAME ($\Delta T_{or} = 1.0°C$) was greater than in sites that had been treated with L-NAME throughout whole body heating. Combined ketorolac + L-NAME treatment (Fig. 2C) attenuated %CVCmax at $\Delta T_{or} \geq 0.4°C$ compared with the control site. There was no difference in %CVCmax after NOS inhibition with L-NAME between ketorolac + L-NAME and control sites. Finally, there was no difference between sites treated with ketorolac and L-NAME (Fig. 2D).

Absolute maximal CVC (28 mM SNP + local heating to 43°C) at control and drug treatment sites are presented in Fig. 3. There were no differences in absolute maximal CVC between the control drug treatment sites ($P = 0.23$).

Table 2. Temperature thresholds for the onset of reflex cutaneous vasodilation

<table>
<thead>
<tr>
<th>Drug Treatment</th>
<th>Absolute $T_{or}$</th>
<th>$\Delta T_{or}$</th>
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<tr>
<td>Control</td>
<td>36.54 ± 0.05</td>
<td>0.31 ± 0.03</td>
</tr>
<tr>
<td>L-NAME</td>
<td>36.70 ± 0.08*</td>
<td>0.48 ± 0.04*</td>
</tr>
<tr>
<td>Ketorolac</td>
<td>36.60 ± 0.10</td>
<td>0.37 ± 0.06</td>
</tr>
<tr>
<td>L-NAME + ketorolac</td>
<td>36.69 ± 0.09*</td>
<td>0.46 ± 0.04*</td>
</tr>
</tbody>
</table>

Values are means $\pm$ SE for control, nitric oxide synthase-inhibited [N-nitro-L-arginine methyl ester (L-NAME)], cyclooxygenase-inhibited (ketorolac), and combination L-NAME + ketorolac sites. Threshold data presented at both absolute $T_{or}$ and $\Delta T_{or}$. Threshold for both L-NAME and combination L-NAME + ketorolac sites occurred at a significantly higher absolute $T_{or}$ and $\Delta T_{or}$ than the control site. *$P < 0.05$ between control and drug-treated sites.

DISCUSSION

The principal findings of this study were as follows. 1) Acute localized COX inhibition with ketorolac increases baseline thermoneutral skin blood flow. These data suggest that COX-derived vasoconstrictors contribute to basal cutaneous vascular tone in middle-aged human skin. Furthermore, concurrent COX and NOS inhibition reduced thermoneutral baseline skin blood flow (similar to control sites), indicating there may be an interaction between these second messenger signaling pathways such that acute COX inhibition increases NO bioavailability. 2) During reflex vasodilation there were no differences between ketorolac-treated and control sites, suggesting either a simple baseline shift increasing skin blood flow throughout whole body heating and/or that in this age group COX-derived vasodilators do not functionally contribute to the increase in skin blood flow with hyperthermia. Finally, because acute...
localized vascular COX inhibition did not attenuate reflex vasodilation in this age group, it is unlikely that low-dose aspirin-induced inhibition of vascular COX is a potential mechanism underlying attenuated reflex vasodilation observed in humans on chronic low-dose aspirin therapy (6).

Normothermia. We found that in middle-aged skin localized non-isoform-specific COX inhibition with ketorolac significantly increased thermoneutral skin blood flow, suggesting that COX-derived vasoconstrictors contribute to basal cutaneous vascular tone in this age group. COX isoforms produce several vasodilators and vasoconstrictors. With primary aging and vascular pathology there is 1) increased COX expression (31) and 2) a functional shift toward COX-derived constricting factors, including endoperoxidases and prostacyclin which stimulate vascular smooth muscle constriction through activation of thromboxane-prostanoid (TP) receptors, contributing to an overall proconstrictor state (31). In aged and hypertensive human vasculature this functional outcome is evidenced by a potentiation in vasodilation to acetylcholine during acute COX inhibition with indomethacin that is mediated by an increase in NO bioavailability (15, 28 –30). Potential mechanisms increasing NO bioavailability during COX inhibition include decreased production of NO scavengers, including superoxide and endoperoxidases (4, 8, 26). Consistent with these findings, in the present study in middle-aged subjects (53 ± 2 yr) the coadministration of the NOS inhibitor L-NAME with ketorolac reduced thermoneutral %CVCmax (vs. ketorolac alone) similar to control sites, suggesting that

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**Fig. 2.** Group mean ± SE cutaneous vascular conductance (CVC) as a percent of maximal response during passive whole body heating. The control site (gray circles in A, B, C), COX-inhibited (ketorolac) site (black circles in A), NOS-inhibited site (open squares in B and D) and combination of L-NAME + ketorolac site (black triangles in C and D) are shown across ΔTof. %CVCmax after NOS inhibition with ΔTof = 1.0°C is also illustrated. A: local treatment with ketorolac augmented %CVCmax compared with control from baseline through ΔTof = 0.3; however, there was no difference between these sites in %CVCmax after NOS inhibition (post-L-NAME) with ΔTof = 1.0°C. B: L-NAME attenuated reflex cutaneous vasodilation throughout whole body heating. %CVCmax after NOS inhibition with ΔTof = 1.0°C in the control site was greater than in sites treated with L-NAME throughout heating. C: combined L-NAME + ketorolac attenuated reflex vasodilation compared with control, and there was no difference in %CVCmax after NOS inhibition with ΔTof = 1.0°C between these sites. D: there was no difference between L-NAME and the combination of L-NAME + ketorolac-treated sites across the rise in oral core temperature. *P < 0.05 (with Bonferroni correction) between control and drug-treated sites.
acute COX inhibition increased NO-dependent vasodilation. We have previously demonstrated a similar increase in \%CVC\textsubscript{max} with acute localized COX inhibition in the cutaneous microvasculature in humans of advanced age (69 ± 1 yr) (7). However, in this older age cohort, combined NOS and COX inhibition did not reduce baseline \%CVC\textsubscript{max} (vs. ketorolac alone) (7), indicating that acute COX inhibition did not increase NO bioavailability in this age group. Collectively, these results suggest that there may be differential regulation of endothelial second messenger pathways along the aging continuum with more significant impairments in NO biosynthetic pathways contributing to reduced endothelial function with more advanced age.

**Reflex vasodilation.** During reflex vasodilation there was not a significant difference in thresholds or \%CVC\textsubscript{max} between ketorolac-treated and control sites (Figs. 1A and 2A). This may be due to a simple baseline shift where differences between the sites at baseline disappear during reflex vasodilation. We originally hypothesized that COX-derived vasodilators would contribute to reflex vasodilation; however, this contribution would be attenuated compared with what has been reported in the literature in young healthy subjects (16). While a baseline effect due to an age-related shift from COX-derived vasodilators toward vasoconstrictors is the simplest potential explanation for the lack of a difference between COX-inhibited and control sites during reflex vasodilation (\(\Delta T_{\text{m}} > 0.4^\circ\text{C}\), there are other potential mechanisms that may underlie this lack of a difference between these sites, including decreased vascular responsiveness to prostacyclin.

Nicholson and colleagues (19) recently demonstrated that primary aging is associated with reduced prostacyclin-mediated vasodilation in the human forearm (intra-arterial infusions with strain-gauge plethysmography). Prostacyclin can act as either a vasodilator or a vasoconstrictor depending on specific vascular smooth muscle receptor activation, where in general TP receptors activation induces vasoconstriction and inositol phosphate (IP) receptor activation induces vasodilation (2). With aging and other vascular pathologies the expression of these vascular smooth muscle receptors shifts to increase TP and reduce IP receptors, respectively (2, 8, 18, 31). In the Nicholson study attenuated prostacyclin-mediated vasodilation in the aged subjects was related to a reduction in endothelial NO generation but not to reduced vascular smooth muscle responsiveness. While we did not directly assess prostacyclin-dependent vasodilation in the skin, this remains a potential explanation for our finding that acute localized COX inhibition did not alter the skin blood flow response in our middle-aged subjects at elevated core temperatures.

In contrast to significant endothelial interactions between prostacyclin- and NO-dependent mechanisms in human skeletal muscle vasculature (19), our data do not suggest a similar interaction between NO and COX pathways during reflex vasodilation in this age group. When we evaluated NO-dependent vasodilation during reflex vasodilation (\(\Delta T_{\text{m}} = 1.0^\circ\text{C}\)) we found that acute COX inhibition did not alter NO-dependent vasodilation (Fig. 2A). This is evidenced by no difference in the reduction in vasodilation due to NOS inhibition when mean body temperature was clamped after a 1.0°C rise (Fig. 2A). In control sites the \%CVC\textsubscript{max} after NOS inhibition was not different from sites treated with ketorolac. However, the control site \%CVC\textsubscript{max} was higher after NOS inhibition than in sites treated with L-NAME throughout whole body heating (Fig. 2B). There are several possible explanations for this finding, including 1) COX-derived vasodilators may contribute minimally to reflex vasodilation, a contribution that is unmasked with NOS inhibition; and 2) the increase in \%CVC\textsubscript{max} (control site) is due to the synergistic role between NO and other putative cotransmitter pathways (33). However, there was no difference between \%CVC\textsubscript{max} after NOS inhibition at the control site compared with sites treated with the combination ketorolac and L-NAME throughout heating, suggesting an upregulation of other endothelium derived hyperpolarization factor (EDHF) mechanisms when both NOS and COX were inhibited at this stage of whole body heating.

**Implications for low-dose aspirin therapy.** We have recently demonstrated that chronic low-dose aspirin therapy consistently and significantly attenuates reflex cutaneous vasodilation in middle aged humans (6). One potential mechanism explaining this finding was that low-dose aspirin was inhibiting vascular COX, thus inhibiting a key enzyme involved in reflex vasodilation (16), even though low-dose aspirin purportedly only inhibits platelet COX-1 (21–23). Our current finding that localized acute non-isosmotic-specific COX inhibition with ketorolac does not attenuate reflex vasodilation in this age group makes it unlikely that vascular COX-derived synthesis of vasodilators is inhibited by low-dose aspirin. Collectively, these two studies suggest a potential role for platelet activation releasing NO, ATP, and/or 5-HT (3, 9, 20) during reflex vasodilation, releasing factors that directly stimulate cutaneous vasodilator pathways. Alternatively, systemic low-dose aspirin therapy may decrease whole blood viscoelastic properties, reducing shear-mediated cutaneous vasodilation through EDHF-dependent pathways (14).

**Limitations.** The aim of the present study was to determine the contribution of COX-dependent vasodilators to reflex cutaneous vasodilation in a middle-aged subject group. We chose to examine this cohort of subjects because of our recent finding that low-dose aspirin therapy significantly attenuates...
reflex vasodilation (6). Because our intent was to examine the cohort of subjects most likely to take low-dose aspirin therapy we did not test a healthy young subject group to replicate the finding of McCord et al. (16). However, we used the same protocol, drug concentrations, and equipment used in that study.

Ketorolac (10 mM) was chosen to nonspecifically inhibit both COX isoforms; thus the specific COX isoform and/or the precise alterations in COX-derived vasoconstrictors and vasodilators are unclear. This concentration has been utilized in our lab (7) and by others and has been shown to efficaciously inhibit COX during normothermic (7, 14) and hyperthermic (16) conditions in young subjects. Although it is unlikely, it is possible that this concentration did not fully inhibit COX in this age group. It is clear that nonspecific COX inhibition augmented baseline and did not attenuate reflex vasodilation. Studies assessing the direct vascular effects of exogenous thromboxanes and prostacyclin with and without appropriate receptor antagonism are necessary.

Summary. In summary we found that localized nonspecific COX inhibition with ketorolac 1) increased baseline %CVCmax, which was attenuated with concurrent NOS inhibition, and 2) did not affect %CVCmax during reflex vasodilation (i.e., there were no differences between ketorolac-treated and control sites). These data suggest an age-related shift from COX-derived vasodilators toward vasoconstrictors that augment baseline skin blood flow through NO-dependent mechanisms. Further, the lack of a difference between these sites during reflex vasodilation may be due to a simple baseline shift and/or may suggest that in this age group vasodilator prostanoids do not functionally contribute to the increase in skin blood flow. Finally, because acute localized vascular COX inhibition did not attenuate reflex vasodilation in this age group, it is unlikely that low-dose aspirin-induced inhibition of vascular COX is a potential mechanism underlying attenuated reflex vasodilation observed in humans taking chronic low-dose aspirin therapy (6).

ACKNOWLEDGMENTS

We are grateful for the technical assistance of Jane Pierzga.

GRANTS

This research was supported by National Institutes of Health Grants R01-AG-07004-18 and M01-RR-10732 (General Clinical Research Center).

REFERENCES


