Influence of hyperoxia on skin vasomotor control in normothermic and heat-stressed humans

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Yamazaki F, Takahara K, Sone R, Johnson JM. Influence of hyperoxia on skin vasomotor control in normothermic and heat-stressed humans. J Appl Physiol 103: 2026–2033, 2007. First published September 20, 2007; doi:10.1152/japplphysiol.00386.2007.—Hyperoxia induces skin vasoconstriction in humans, but the mechanism is still unclear. In the present study we examined whether the vasoconstrictor response to hyperoxia is through activated adrenergic function (protocol 1) or through inhibitory effects on nitric oxide synthase (NOS) and/or cyclooxygenase (COX) (protocol 2). We also tested whether any such vasoconstrictor effect is altered by body heating. In protocol 1 (n = 11 male subjects), release of norepinephrine from adrenergic terminals in the forearm skin was blocked locally by iontophoresis of bretylium (BT). In protocol 2, the NOS inhibitor Nω-nitro-L-arginine methyl ester (L-NAME) and the nonselective COX antagonist ketorolac (Keto) were separately administered by intradermal microdialysis in 11 male subjects. In the two protocols, subjects breathed 21% (room air) or 100% O2 in both normothermia and hyperthermia. Skin blood flow (SkBF) was monitored by laser-Doppler flowmetry. Cutaneous vascular conductance (CVC) was calculated as the ratio of SkBF to blood pressure measured by Finapres. In protocol 1, breathing 100% O2 decreased (P < 0.05) CVC at the BT-treated and at untreated sites from the levels of CVC during 21% O2 breathing both in normothermia and hyperthermia. In protocol 2, the administration of L-NAME inhibited (P < 0.05) the reduction of CVC during 100% O2 breathing in both thermal conditions. The administration of Keto inhibited (P < 0.05) the reduction of CVC during 100% O2 breathing in hyperthermia but not in normothermia. These results suggest that skin vasoconstriction with hyperoxia is partly due to the decreased activity of functional NOS in normothermia and hyperthermia. We found no significant role for adrenergic mechanisms in hyperoxic vasoconstriction. Decreased production of vasodilator prostaglandins may play a role in hyperoxia-induced cutaneous vasoconstriction in heat-stressed humans.

Skin vasomotor control is modified by various nonthermal factors such as the baroreflex, dehydration, or dynamic exercise (22). An increase of arterial O2 tension (PaO2), another nonthermal factor, induces peripheral vasoconstriction that includes the cutaneous circulation (8, 10, 47, 48). Because the sympathetic nerve activity to muscle is decreased or not changed by hyperoxic conditions (23, 41), this peripheral vasoconstriction may be associated with nonsympathetic mechanisms, but it is unknown whether this speculation is applicable to cutaneous vasomotor control. Findings from previous studies suggest that PaO2 influences the endothelium-derived factors that contribute to the maintenance of vascular tone (34, 35). In vitro, superoxide anions derived from hyperoxia react rapidly with nitric oxide (NO) (38). In rat cremaster arterioles, removal of the endothelium or inhibition of prostaglandin (PG) synthesis eliminated the vasoconstrictor response to increased PaO2 (34). Additionally, exposure of human umbilical arteries to hyperoxia resulted in a 30% inhibition of the ability of the vessels to produce PGs (43). Thus hyperoxia may impair the function of endothelium-derived vasoactive factors, such as NO and PGs. However, there is no direct information available about the extent to which those mechanisms mediate the skin vasomotor response to hyperoxia in humans.

The mechanisms by which hyperoxia affects vasomotor control of the cutaneous circulation may differ between normothermia and hyperthermia. In normothermia, adrenergic sympathetic mechanisms are predominant in the control of skin blood flow (SkBF), whereas in hyperthermia, nonadrenergic active vasodilator mechanisms become predominant in that control (22). In hyperthermia, cutaneous active vasodilation is thought to act via a cholinergic cotransmitter system (29). Importantly, Kellogg et al. (28) reported that acetylcholine-induced vasodilation is mediated by NO and PGs in human skin. In line with these findings, McCord et al. (33) recently reported that PGs contribute to cutaneous active vasodilation. Thus hyperthermia creates the situation in which one or more vasodilator PGs are brought into play and, consequently, may be more liable for antagonism by hyperoxia. Furthermore, the cutaneous active vasodilator process has been shown to include a role for NO synthase (NOS) (42). If NO and/or PG mechanisms play roles in hyperoxic vasoconstriction in skin, then those roles may be more significant in hyperthermia in which the cholinergic system is activated.

It has been suggested that active vasodilation is linked to sweating activity (30, 31, 44). The issue of the relationship of active vasodilation and sweating activity has not been resolved in humans, but it is important to determine effects of hyperoxia on both sweating and the cutaneous circulation because, if they are functionally linked, comparison of sudomotor and vasomotor responses may provide further understanding of the mechanisms of hyperoxic skin vasoconstriction in hyperthermia.

In this study, we investigated the effects of hyperoxia on the skin vasomotor control during normothermia and hyperthermia. First, we tested the hypothesis that the vasoconstrictor response during O2 breathing is through activated adrenergic vasoconstrictor function (protocol 1). In this protocol, the

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effect of hyperoxia on sweating was also examined. In protocol 2, we tested the hypothesis that the vasoconstrictor response to hyperoxia is through inhibitory effects on NO- or PG-dependent mechanisms. We also hypothesized that the inhibitory effects on NO- or PG-dependent mechanisms with hyperoxia are more significant in hyperthermia because of the increased roles of those systems in the cutaneous active vasodilator process.

**METHODS**

**Subjects**

A total of 22 male volunteers participated in the experiments. Their average age was 23 ± 1 (SE) yr, average weight was 67 ± 2 kg, and average height was 173 ± 1 cm. All subjects were healthy nonsmokers with no history of cardiovascular disease. Written informed consent was obtained after a thorough explanation of the present study, including its purpose and risks. The experiments were approved by the Ethics Committee of Medical Care and Research of the University of Occupational and Environmental Health.

**Measurements**

SKBF was monitored continuously with laser-Doppler flowmeters (ALF21, Advance, Tokyo, Japan). The blood flow measurements are specific to the skin and are not influenced by blood flow to underlying skeletal muscle (40). As an index of core temperature, esophageal temperature specific to the skin and are not influenced by blood flow to underlying (ALF21, Advance, Tokyo, Japan). The blood flow measurements are calculated (45). Heart rate (HR) was determined from the electrocardiogram. Mean arterial pressure (MAP) was measured continuously from a cuff on the middle finger (Finapres, Ohmeda, Madison, WI). Arterial O2 saturation (SaO2) was measured from the index finger with a pulse oximeter (Biox3740, Ohmeda, Louisville, CO). Minute ventilation, end-tidal O2 (PetO2) and end-tidal CO2 (PetCO2) were analyzed using a breath-by-breath gas analyzer (RL-600, Westron, Chiba, Japan). Local sweating rates (SR) were measured by the ventilated capsule method. The sweat capsules were located on the ventral surfaces of the right and left forearm. Dry air was supplied to the sweat capsules (5.7-cm2 area) at the rate of 1.0 L/min. The humidity of the air flowing out of the capsules was measured with capacitance hygrometers (HMP 133Y, Vaisala, Helsinki, Finland). The measured variables were recorded by a data logger (DE1200 universal, NEC Sanei, Tokyo, Japan).

**Experimental Procedures and Protocols**

The experiments consisted of two different protocols, each conducted under constant environmental conditions (ambient temperature; 26 ± 0.5°C, relative humidity; 50 ± 5%). Each subject participated in one experiment. The protocols are as follows.

**Protocol 1.** Effect of hyperoxia on adrenergic cutaneous vasoconstrictor function. Eleven subjects participated in this experiment. To examine the effect of hyperoxia on adrenergic vasoconstrictor function in the skin, SKBF was monitored at three skin sites: bretylium (BT)-treated and untreated sites on the ventral surface of the left forearm and an untreated site on the left palm. BT (Sigma, St. Louis, MO), which blocks the release of norepinephrine and cotransmitters from adrenergic terminals (14), was applied iontophoretically to a 0.64-cm2 area of skin (26). This method produces a selective local blockade of the cutaneous adrenergic vasoconstrictor system lasting 3 h or more (26). The iontophoresis was applied at 400 μA/cm2 for 10 min on the forearm. This is only modestly successful for glabrous skin (21) and therefore was not applied to palmar areas in the present study. Approximately 60 min after the application of BT, the subject dressed in a tube-lined water-perfused suit that covered the entire body except for the head and arms. Each subject rested in the supine position for ~30 min. During this period, a finger cuff for measuring arterial pressure, thermocouples, flow probes, probe holders for controlling local Tsk, and sweat capsules were applied. Mean Tsk was controlled by changing the temperature of the water perfusing the suit. The local temperature of the forearm areas (6.3 cm2) surrounding the laser-Doppler probe was controlled by a local temperature controller (model 888, Schoelertec, Osaka, Japan). Local Tsk was not controlled for the palm sites as the size and shape of the local temperature controllers do not conform to the palm. Mean Tsk and local Tsk for the forearm sites were maintained at 34–35°C. Whole body cold stress (3-min duration, aggressive cooling) was induced by perfusing the suit with cold water to lower whole body Tsk quickly from 34–35°C to 30–32°C to test for an adequate blockade of the vasoconstrictor nerves (26). After recovery from this period of cooling, subjects breathed room air (21% O2) or 100% O2 through a two-way nonrebreathing valve (model 7900, Hans Rudolph, Kansas City, MO). The inspiratory side of the valve was connected to Douglas bags containing 100% O2 or open to room air via a three-way stopcock. Subjects breathed through the mask for 15 min. Measurements for a 3-min baseline control period were then performed during breathing room air followed by 100% O2 breathing for 5 min. If necessary, CO2 was added to the inspired gas during breathing 100% O2 to hold the end-tidal CO2 levels constant (i.e., isocapnic). On completion of the test at normothermia, mean Tsk was increased to 38–39°C by perfusing the suit with 46–49°C water. Mean Tsk reached the targeted range after ~10 min, and it was maintained at that level for 35–45 min before the baseline control measurements in hyperthermia. After Tes and HR stabilized (changes <0.05°C or 5 beats/min, respectively, over 5 min), measurements were made during normoxia and hyperoxia as in the normothermic condition. After whole body heating, mean Tsk was returned to normothermic levels. Finally, the maximal level of cutaneous vascular conductance (CVC) was determined by local warming of the areas of forearm skin around the laser Doppler probes to 42°C for 30–40 min (45).

**Protocol 2.** Roles of NOS and cyclooxygenase (COX) in hyperoxic cutaneous vasoconstriction. This protocol was performed to test the involvement of NO- and/or PG-dependent mechanisms in the skin vascular response to hyperoxia during normothermia and hyperthermia. Eleven subjects were studied in this protocol. To antagonize the generation of NO by NOS and the generation of PGs by COX, the NOS inhibitor [N5-nitro-l-arginine methyl ester (l-NAME); Sigma] and the COX inhibitor ketorolac [(Keto); Sigma] were separately delivered by intradermal microdialysis. This permitted local and continuous delivery of these drugs to the cutaneous interstitium at high concentrations without confounding systemic effects (27, 28). Each microdialysis probe consisted of a microdialysis membrane with an 18-kDa cutoff (Spectrum, Laguna Hills, CA), 1 cm in length and 200 μm in diameter, connected to polyimide tubing with a 0.0049-in. internal diameter. The probe was reinforced with 0.0015-in.-diameter coated stainless steel wire (27, 50).

Subjects had three intradermal microdialysis probes placed within the dermis of the ventral left forearm. Before microdialysis probe insertion, cold packs were applied to the ventral surface of the left forearm as a temporary anesthetic. Needles (25 gauge) were inserted intradermally into the arm for ~2.5 cm. The probes were then fed through the lumen of the needle. Probes were aligned such that the microdialysis membranes were centered within the dermis. The needles were then removed, leaving the probes in place. After insertion of microdialysis probes, subjects waited ~2.5 h to allow insertion trauma to resolve.

After resolution of insertion trauma, sterile saline was perfused (4 μl/min) at all three sites. SKBF was monitored using SKBF probes placed directly over the center of microdialysis probes. Data collection began with a baseline period of 15–20 min during saline infusion. Subsequently, one site received 20 mM l-NAME (l-NAME site)
dissolved in saline. This concentration of L-NAME has been reported to produce a complete NOS inhibition (27, 50). A second site received 10 mM Keto (Keto site) dissolved in saline. This concentration of Keto was based on findings from an earlier study in which 10 mM Keto was the greatest concentration that caused no consistent increase in baseline SkBF (28). The third site received saline (control site). This phase of the experiment lasted 40–60 min, until a steady-state blood flow was reached. As in protocol 1, measurements during a 3-min control period were performed while breathing room air, followed by breathing 100% O2 for 5 min during normothermia. As in protocol 1, this sequence was repeated during whole body heating. Tsk was then returned to normothermic levels, and all microdialysis probes were perfused with 28 mM nitroprusside (sodium nitroprusside; Sigma) in saline for 25–40 min to cause maximal vasodilation (25, 27). This method to dilate cutaneous vessels to maximal levels produces results not different from those achieved by raising the local Tsk to 42°C (27).

Data Processing and Statistical Analysis

The measured variables were sampled each 5 s and averaged over 1-min intervals. CVC was calculated from the ratio of SkBF to MAP. Values for SR from the two forearm sites were averaged for each subject. The changes in CVC were expressed as percentages of maximal values for the forearm sites or as percent changes from the normothermic and normoxic baseline values for the palmar sites. The normoxic baseline data were averaged over the 3-min control period. Hyperoxic data were averaged over the last 2 min of the periods of 100% O2 breathing.

Effects of hyperoxia and whole body heating on changes in each variable were evaluated using two-way repeated-measures ANOVA (Figs. 1 and 2, Tables 1 and 2). Effects of drug administration on baseline CVC values and the magnitude of changes in CVC by hyperoxia or whole body heating were evaluated using one-way ANOVA. For all ANOVAs, the Student-Newman-Keuls test was used to determine where significant differences occurred. Effects of cold stress on CVC values were evaluated using Student’s paired t-test. *P < 0.05 was considered significant.

RESULTS

Protocol 1

Whole body cooling decreased (P < 0.001) CVC at the untreated sites in the forearm from 9.6 ± 1.3% to 5.5 ± 0.7% of maximum CVC. Cold stress also decreased (P < 0.001) CVC at the untreated palmar site by 86.3 ± 2.4% from the normothermic baseline level. CVC at BT-treated sites in the forearm was not significantly altered by the cold stress (from 11.4 ± 1.1% to 10.7 ± 1.1% of maximum CVC; P = 0.14), verifying that BT treatment was effective in blocking adrenergically mediated cutaneous vasoconstriction.

Breathing 100% O2 increased (P < 0.001) SaO2 and PETO2 during both normothermia and heat stress (Fig. 1, Table 1), whereas PETCO2 was not altered (P > 0.10) during breathing 100% O2 in either thermal condition (Table 1). Whole body
heating led to increases in mean $T_{sk}$ and $T_{es}$ ($P < 0.001$), but these temperature variables were not altered ($P > 0.38$) by breathing 100% $O_2$ under either normothermic or heat-stress conditions (Table 1). Whole body heating increased ($P < 0.001$) CVC at the BT-treated sites by 38.5 ± 7.0% of maximum CVC and at untreated sites by 42.1 ± 5.2% of maximum CVC from normothermic baseline levels. Breathing 100% $O_2$ decreased ($P < 0.05$) CVC at the untreated sites in the forearm and palm under both normothermic and heat-stress conditions. BT treatment did not abolish the reduction in CVC during 100% $O_2$ breathing under the two thermal conditions. The hyperoxia-induced reductions of CVC did not differ between the BT-treated and untreated sites during normothermia ($-0.7 ± 0.3%$ of maximum CVC at untreated site, $-0.8 ± 0.2%$ of maximum CVC at BT-treated site; $P = 0.90$) or during heat stress ($-4.8 ± 2.4%$ of maximum CVC at untreated site, $-4.6 ± 3.1%$ of maximum CVC at BT-treated site; $P = 0.88$). Breathing 100% $O_2$ did not change ($P > 0.20$) forearm SR from the normoxic baseline level (Fig. 1). The $O_2$ breathing decreased ($P < 0.001$) HR during normothermic as well as hyperthermic conditions but did not alter MAP in either of the two thermal conditions (Table 1).

**Protocol 2**

In normothermia, CVC did not differ ($P = 0.09$) among the three sites during the baseline control period after the administration of the antagonists. As in protocol 1, breathing 100% $O_2$ increased ($P < 0.001$) $S_{aO_2}$ during normothermia and heat stress (Fig. 2), but did not alter $T_{es}$ or mean $T_{sk}$ (Table 2). In

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**Table 1. Physiological variables during 21% and 100% $O_2$ breathing under normothermic and heat-stressed conditions in Protocol 1**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normothermia</th>
<th>Heat Stress</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{es}$, °C</td>
<td>36.72 ± 0.07</td>
<td>36.71 ± 0.07</td>
</tr>
<tr>
<td>Mean $T_{sk}$, °C</td>
<td>33.94 ± 0.14</td>
<td>34.06 ± 0.14</td>
</tr>
<tr>
<td>$V_E$, l/min</td>
<td>8.1 ± 0.4</td>
<td>9.7 ± 0.7*</td>
</tr>
<tr>
<td>RR, breaths/min</td>
<td>15.6 ± 0.8</td>
<td>16.5 ± 0.9*</td>
</tr>
<tr>
<td>$P_{ETCO_2}$, mmHg</td>
<td>41.8 ± 0.8</td>
<td>41.7 ± 0.8</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>78.5 ± 1.8</td>
<td>78.7 ± 1.4</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>56.0 ± 2.8</td>
<td>53.3 ± 2.7*</td>
</tr>
<tr>
<td>Forearm CVC untreated, %max</td>
<td>9.0 ± 1.1</td>
<td>8.3 ± 1.1*</td>
</tr>
<tr>
<td>Forearm CVC BT treated, %max</td>
<td>10.4 ± 0.7</td>
<td>9.6 ± 0.8*</td>
</tr>
<tr>
<td>Palmar CVC untreated, %baseline</td>
<td>100</td>
<td>75.6 ± 8.4*</td>
</tr>
<tr>
<td>Forearm SR, mg·cm$^{-2}$·min$^{-1}$</td>
<td>0.06 ± 0.01</td>
<td>0.06 ± 0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE. $T_{es}$, esophageal temperature; $T_{sk}$, skin temperature; $V_E$, ventilation; RR, respiratory rate; $P_{ETCO_2}$, end-tidal $CO_2$; MAP, mean arterial pressure; HR, heart rate; CVC, cutaneous vascular conductance; SR, sweating rate; BT, bretylium; %max, % of maximum CVC; %baseline, % of baseline CVC. *$P < 0.05$ vs. 21% $O_2$. †$P < 0.05$ vs. normothermia.
normothermia, hyperoxia decreased ($P < 0.001$) CVC at all sites; however, the decrease of CVC at L-NAME treated sites was smaller ($P < 0.05$) than at saline- or Keto-treated sites. The hyperoxia-induced reductions of CVC did not differ ($P = 0.36$) between saline and Keto treatments. Whole body heating increased ($P < 0.0001$) CVC at all sites; the increase of CVC (by $18.2 \pm 3.5\%$ of maximum CVC) at L-NAME-treated sites from the normothermic level was significantly less ($P < 0.001$) than with saline (an increase of $46.3 \pm 3.7\%$ of maximum CVC) or Keto treatment (increase of $38.4 \pm 5.1\%$ of maximum CVC) (Fig. 2, Table 2). The increase of CVC at Keto-treated sites with body heating was less ($P = 0.03$) than with saline treatment. In hyperthermia, breathing $100% O_2$ decreased CVC at all sites; but the reductions of CVC at L-NAME- and Keto-treated sites were significantly less ($P < 0.05$) than that at saline-treated sites (Fig. 3).

**DISCUSSION**

There were several major findings from the present study: 1) hyperoxia induces vasoconstriction in both nonglabrous and glabrous skin, 2) pharmacological blockade of adrenergic vasoconstrictor nerve does not abolish or noticeably affect the hyperoxia-induced vasoconstriction in either normothermia or hyperthermia, 3) inhibition of functional NOS decreases the hyperoxic vasoconstrictor responses in both thermal conditions, 4) COX inhibition did not alter the vasoconstrictor response in normothermia but decreases the response in hyperthermia, and 5) sweating activity was not influenced by hyperoxia.

It is generally agreed that glabrous skin lacks influence from active vasodilator nerves (21, 49). Therefore, reflex control of SkBF in these regions is thought to be controlled entirely by the noradrenergic vasoconstrictor system. There are reasons to anticipate that system to be stimulated by hyperoxia. $O_2$ breathing induces vasoconstriction in glabrous skin. Although the influence of hyperoxia on the sympathetic nerve activity to skin is unknown, there are reports that muscle sympathetic nerve activity is decreased under hyperoxic conditions (17, 20, 23, 41) and is increased under hypoxic conditions (16, 17, 23, 46). In contrast to the anticipated effects of sympathetic activity on vasomotion, blood flow measurements by venous occlusion plethysmography or dye dilution indicate that the limb vasculature is constricted in hyperoxia (5, 32, 47) and dilated in hypoxia (46). Laser-Doppler measurements of SkBF in humans indicate that hyperoxia ($SaO_2 = 99.8\%$) induces cutaneous vasoconstriction (48), whereas hypoxia ($SaO_2 = \sim 85\%$) tends to vasodilate the skin (46). The hypoxic vasodilator response in forearm skin was insensitive to combined $\alpha$- and $\beta$-adrenergic blockade but was diminished by NOS inhibition following combined $\alpha$- and $\beta$-adrenergic blockade (46). The present finding that the hyperoxic reduction of CVC at BT-treated sites did not differ from that at untreated sites in normothermia or hyperthermia suggests the mechanism for the hyperoxic vasoconstriction is also nonadrenergic. Taken together, these findings make it doubtful that hyperoxia-induced vasoconstriction in the skin is of adrenergic origin. This makes it equally unlikely that the vasoconstriction is of reflex origin, including chemoreceptor-mediated reflexes.

What is the nonadrenergic mechanism for hyperoxic skin vasoconstriction? Previous studies in experimental animals suggest that $PaO_2$ influences the endothelium-derived factors that contribute to the maintenance of vascular tone in cremaster muscle and heart (34, 35). In human studies, hyperoxia attenuated endothelium-dependent vasodilation in forearm skin (48). Moreover, it has been suggested that hyperoxia-derived free radicals impair the activity of endothelium-derived vasoactive factors in the forearm (32). Thus there is evidence that endothelium-derived factors contribute to the nonadrenergic mechanisms of hyperoxic vasoconstriction. The findings from the present study support that conclusion. Hence, it may be that hyperoxia decreases the production and/or bioavailability of NO in the skin in both normothermic and heat-stressed humans.
The previous findings from rat cremaster arterioles and human umbilical arteries also suggest that PGs, another class of endothelium-derived vasoactive factors, contribute to hyperoxic vasoconstriction (34, 43). Our data suggest the possibility of involvement of PGs in this response in hyperthermia, but the evidence does not support such a role in normothermia. The reason for this inconsistency in results between normothermic and hyperthermic conditions is unclear, but it may be that the roles of PGs in the control of SkBF would differ between the two thermal conditions. Kellogg et al. (28) observed that intradermal infusion of Keto at higher concentrations (>10 mM) caused a progressive increase in SkBF in normothermia. Furthermore, 10 mM Keto significantly increased baseline CVC in older subjects but not young subjects in normothermia (19). These observations suggest that PGs can act as tonic vasoconstrictor substances in the skin in normothermia. In contrast, treatment with Keto inhibited heat-stress induced vasodilation, suggesting that PGs contribute to the active vasodilator response in skin in hyperthermia (33). As mentioned above, because the neural control of SkBF differs between normothermia and hyperthermia (22, 29), the involvement of PGs in the stress-induced reactivity of skin vessels might be altered by thermal status. It is possible therefore that different involvement of PGs in cutaneous vasomotor control between hyperthermia and normothermia is responsible for the different effects of COX inhibition on skin vasomotor response during O2 breathing. This suggests that those PGs produced as part of the cutaneous active vasodilator process (33) are affected by hyperoxia.

Application of l-NAME or Keto inhibited but did not abolish the reduction of CVC with O2 breathing. This could be due either to incomplete inhibition of NOS or COX by the antagonists at the concentrations we used or due to the involvement of mechanisms other than NOS- or COX-mediated pathways. It has been reported that hyperoxia stimulates increased production of the endothelium-derived vasconstrictor endothelin in human retina (7). Furthermore, hyperoxia may cause vasoconstriction through an effect on ATP-sensitive K+ channels (35) because it has been demonstrated that ATP-sensitive K+ channels play an important role in mediating hypoxic vasodilation (9). In the present study, the sum of the reductions in CVC with hyperoxia at l-NAME- and Keto-treated sites would roughly equal that at the control sites (Fig. 3). However, it has been reported that the COX pathway appears to work independently of NO in cutaneous active vasodilation in human skin (33). In light of those earlier observations and the present findings, it would be interesting to test whether combined NOS and COX antagonism would completely block the hyperoxia-induced vasodilation as a means of distinguishing among these possibilities.

In protocol 1, the vasoconstriction in glabrous (palmar) skin was greater than in nonglabrous (forearm) skin. Reasons for this difference are unclear, but do follow a general pattern of greater responsiveness in glabrous skin to external stimuli, perhaps of a behavioral origin (36, 39). Also, bursts of skin sympathetic nerve are loosely coupled to the resting respiratory pattern (4, 6, 15). Isocapnic hyperoxia increases ventilation by several mechanisms, including the Haldane effect in normothermic humans (2, 3). The hyperoxia-induced hyperventilation also occurred in hyperthermia, which is another stimulation for hyperventilation (13, 18). It is possible that hyperoxia-induced hyperventilation serves to stimulate a cutaneous vasoconstriction in palm rather than forearm in normothermia and hyperthermia. Arteriovenous Anastomoses are much more abundant in glabrous skin (36). It could also be that anastomotic vessels are more sensitive to the nonreflex vasoconstrictor effects of hyperoxia. It is not currently possible to distinguish between these possible mechanisms for this difference in response between glabrous and nonglabrous skin to hyperoxia.

It has been proposed that cutaneous active vasodilation is produced by the action of vasodilator substances released from activated sweat glands or cholinergic postganglionic sympathetic nerves (12, 30, 31, 44). Although there is not uniform agreement as to whether sweating and cutaneous active vasodilation are causally linked (22, 24); if they are, these data suggest that withdrawal of active vasodilator activity is not the source of the vasoconstrictor response to hyperoxia because SR was unchanged during hyperoxia. They also suggest that there is not a central change in control of thermoregulatory effectors with hyperoxia.

O2 breathing is widely used under therapeutic and experimental situations in medicine. For example, O2 breathing in a hyperbaric environment (hyperbaric O2 therapy) has been used as an adjunct for management of a variety of pathologies, including extremity trauma, cancer, and gas embolism (1, 11). Therefore the effects of breathing O2-enriched gas mixtures on physiological function should be considered for these patients. It is suggested, however, that the effect of hyperoxia on heat dissipation is minimal in this experimental setting, because hyperoxia did not significantly change core temperature or Tsk. The absence of a measurable change in Tsk is in keeping with

![Fig. 4. Model for vasoconstrictor response to hyperoxia in human skin. Influence of hyperoxia on skin vasomotion is through both reflex and nonreflex pathways, the nonreflex pathways are usually dominant. During normothermia, the nonreflex vasoconstriction with hyperoxia occurs, at least, through inhibitory effect on basal NOS activity in the skin. During hyperthermia in which cholinergic system is reflexly activated throughout thermoregulatory center, the hyperoxic vasoconstrictor response is through inhibitory effects on nitric oxide synthase (NOS) and cyclooxygenase (COX)-dependent mechanisms. Sweating is activated by hyperthermia but not by hyperoxia. Inhibitory stimuli.](http://jap.physiology.org/)

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the relatively small, but significant changes in CVC with hyperoxia. One of the benefits of the response may be to limit the delivery of O$_2$ and of free radical formation.

Figure 4 shows our working model of the potential mechanisms by which hyperoxia exerts its effects on the skin circulation as indicated by this study. Influence of hyperoxia on skin vessel includes reflex and nonreflex components. Blockade of functional adrenergic nerves with BT did not diminish the vasocostrictor response. Although there is actually not a consensus as to the relationship between the active vasodilator system and sudomotor control, hyperoxia did not change sweating activity. These findings suggest that the reflex components are not major pathways for hyperoxia-induced vasocostriction in the skin. In normothermia, the nonreflex vasocostriction during hyperoxia occurs, at least in part, through inhibitory effects on basal NOS activity. In hyperthermia in which the active vasodilator system is engaged, the hyperoxic vasocostrictor response is through inhibitory effects on NOS and COX-dependent mechanisms. These mechanisms are activated by release of acetylcholine and cotransmitters from postganglionic sympathetic nerves, a part of the cutaneous active vasodilator process.

Rousseau et al. (37) recently reported that hyperoxia-induced vasocostriction was observed only in areas of skin with relatively high blood flow. The results from that study and ours are in agreement that hyperoxia induces cutaneous vasocostriction when blood flow is elevated. The results differ with lower initial levels of blood flow: we found a small but significant vasocostriction, whereas none was observed in the study by Rousseau and colleagues. We cannot ascertain the reasons for this difference as protocol, and instrumental differences preclude clear comparison. For example, it is not known whether the sensitivity of blood flow measurement by Laser-Doppler scanners is similar to that of the single-fiber probes used here or whether the laboratory conditions differed sufficiently (23°C in the study by Rousseau et al. vs. 26°C in the present study) to account for the observed difference in response. However, because the finding suggests the baseline levels of CVC influence the vasocostrictor response, we cannot conclude that the sensitivity to hyperoxia is necessarily affected by hyperthermia. Administration of l-NAME decreased the baseline levels of CVC in hyperthermia (Fig. 2). It is possible that the decrease of baseline CVC values at the l-NAME site decreased the effect of hyperoxia on the vasocostrictor response at that site. Further study is required to find to what extent the vasocostrictor response is influenced by the baseline level of CVC.

In conclusion, these findings suggest that hyperoxia acts to constrict the cutaneous vasculature in both palm and forearm. In the forearm, this involves an NO-dependent pathway but not adrenergic function. The involvement of PG-mediated pathways in the hyperoxic vasocostriction appears to depend on thermal status.

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