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 normothermic and hyperthermic. 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. Increased 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 non-smokers 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 (Tes) was measured with a polyethylene-sealed thermocouple specific to the skin and are not influenced by blood flow to underlying (ALF21, Advance, Tokyo, Japan). The blood flow measurements are not influenced by blood flow to underlying (ALF21, Advance, Tokyo, Japan). The blood flow measurements are not influenced by blood flow to underlying (ALF21, Advance, Tokyo, Japan). The blood flow measurements are not influenced by blood flow to underlying.
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 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. 

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 Tsk and Tes (P < 0.001), but these temperature variables were not altered (P > 0.38) by breathing 100% O2 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% O2 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% O2 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% O2 did not change (P > 0.20) forearm SR from the normoxic baseline level (Fig. 1). The O2 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% O2 increased (P < 0.001) SaO2 during normothermia and heat stress (Fig. 2), but did not alter Tsk or mean Tsk (Table 2).
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 ± 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 ± 3.7% of maximum CVC) or Keto treatment (increase of 38.4 ± 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% O2 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. O2 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 hypoxic 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 (Sao2 = 99.8%) induces cutaneous vasoconstriction (48), whereas hypoxia (Sao2 = ~85%) tends to vasodilate the skin (46). The hypoxic vasodilator response in forearm skin was insensitive to combined α- and β-adrenergic blockade but was diminished by NOS inhibition following combined α- and β-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 activity.

What is the nonadrenergic mechanism for hyperoxic skin vasoconstriction? Previous studies in experimental animals suggest that Pao2 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 humans (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/content/103/4/2031/F4)

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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 O₂ 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 vasoconstrictor 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 vasoconstriction in the skin. In normothermia, the nonreflex vasoconstriction during hyperoxia occurs, at least in part, through inhibitory effects on basal NO activity. In hyperthermia in which the active vasodilator system is engaged, the hyperoxic vasoconstrictor 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 vasoconstriction was observed only in areas of skin with relatively high basal blood flow. The results from that study and ours are in agreement that hyperoxia induces cutaneous vasoconstriction when blood flow is elevated. The results differ with lower initial levels of blood flow: we found a small but significant vasoconstriction, 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 vasoconstrictor response, we cannot conclude that the sensitivity to hyperoxia is necessarily affected by hyperthermia. Administrations 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 vasoconstrictor response at that site. Further study is required to find to what extent the vasoconstrictor 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 vasoconstriction appears to depend on thermal status.

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