Systemic hypoxia causes cutaneous vasodilation in healthy humans

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Submitted 20 December 2006; accepted in final form 15 May 2007

Regional hypoxia causes cutaneous vasodilation in healthy humans

J Appl Physiol 103: 608–615, 2007. First published May 17, 2007; doi:10.1152/japplphysiol.01443.2006.—Hypoxia and hypercapnia represent special challenges to homeostasis because of their effects on sympathetic outflow and vascular smooth muscle. In the cutaneous vasculature, even small changes in perfusion can shift considerable blood volume to the periphery and thereby impact both blood pressure regulation and thermoregulation. However, little is known about the influence of hypoxia and hypercapnia on this circulation. In the present study, 35 healthy subjects were instrumented with two microdialysis fibers in the ventral forearm. Each site was continuously perfused with saline (control) or bretylium tosylate (10 mM) to prevent sympathetically mediated vasoconstriction. Skin blood flow was assessed at each site (laser-Doppler flowmetry), and cutaneous vascular conductance (CVC) was calculated as red blood cell flux/mean arterial pressure and normalized to baseline. In 13 subjects, isocapnic hypoxia (85 and 80% O2 saturation) increased CVC to 120 ± 10 and 126 ± 7% baseline in the control site (both P < 0.05) and 113 ± 3 (P = 0.087) and 121 ± 4% baseline (P < 0.05) in the bretylium site. Adrenergic blockade did not affect the magnitude of this response (P > 0.05). In nine subjects, hyperpnea (matching hypoxic increases in tidal volume) caused no change in CVC in either site (both P > 0.05). In 13 subjects, hypercapnia (+5 and +9 Torr) increased CVC to 111 ± 4 and 111 ± 4% baseline, respectively, in the control site (both P < 0.05), whereas the bretylium site remained unchanged (both P > 0.05). Thus both hypoxia and hypercapnia cause modest vasodilation in nonacral skin. Adrenergic vasoconstriction of neural origin does not restrain hypoxic vasodilation, but may be important in hypnic vasoconstriction.

sympathetic nervous system; vascular resistance; microdialysis; skin; altitude; sleep apnea syndrome

REGULATION OF SKIN BLOOD FLOW represents a complex scheme of neural and nonneural vasoconstrictor and vasodilator signals controlled by multiple homeostatic mechanisms (e.g., thermoregulatory reflexes, baroreflexes) (22). While the effects of hypoxia and hypercapnia on skin blood flow are poorly understood, it has been suggested that both stimuli cause vasodilation in human nonacral skin (10, 28, 39, 41). If this is so, then conditions characterized by low oxygen or high carbon dioxide levels (e.g., altitude, respiratory disease) may cause peripheral shifts in blood volume due to the high compliance of the cutaneous vasculature. In this setting, thermoregulation and/or blood pressure regulation may be challenged.

The evidence suggesting that carbon dioxide dilates cutaneous vessels was collected before modern methods of cutaneous perfusion measurement. For example, subcutaneous injection of gas containing 12.5–100% carbon dioxide causes a widespread increase in forearm skin temperature (10). Furthermore, immersion of the hand in water saturated with carbon dioxide results in an increase in the rate of heat elimination to the water bath, while immersion of the calf causes an increase in limb blood flow of up to 150% (9, 39). Thus a body of evidence, albeit indirect, indicates that cutaneous vessels dilate in response to carbon dioxide. Interestingly, studies of forearm hemodynamics during systemic hypercapnia indicate that local vasodilation to carbon dioxide may be partially offset by sympathetic vasoconstriction mediated through chemoreceptor activation (30). Therefore, the net hemodynamic response in the skin may also depend on the relative activation of vasoconstrictor and vasodilator signals.

During hypoxia, blood flow to the hand and fingers decreases (11, 26). These observations lead to the belief that hypoxia causes cutaneous vasoconstriction, because the hand and finger circulations are directed mostly to skin. However, both the structure and autonomic innervation of the cutaneous circulation in the hand are different than that of the hairy (nonacral) skin that covers most of the body surface (33). Therefore, the vascular response to hypoxia in nonacral skin may be different from the responses in the hands and fingers. Along these lines, Rowell et al. (34) measured the effect of hypoxia on forearm vascular responses to exercise using venous occlusion plethysmography. The relationship between forearm blood flow and esophageal temperature was not altered by hypoxia, suggesting that hypoxia did not stimulate vasoconstriction directed to the skin. Importantly, forearm blood flow measurement using venous occlusion plethysmography excludes circulation to the hand through inflation of a wrist cuff to suprasystolic pressure. The resulting blood flow measurement reflects perfusion of nonacral forearm skin plus underlying skeletal muscle.

Our laboratory previously published initial observations of cutaneous vasodilation during hypoxia in nonacral skin when α-adrenergic vasoconstriction was blocked with brachial artery infusion of phentolamine (41). However, these measurements were taken in only a subset of subjects (n = 6) in a study focused on skeletal muscle vascular regulation. While forearm vascular responses were distinctly greater when α-adrenergic vasoconstriction was blocked, cutaneous vascular responses were variable, and differences between control and blockade responses were not assessed (see discussion in Ref. 41). Furthermore, no attempt was made to control for ventilation in that study. In light of documented changes in cutaneous sympathetic outflow during hyperventilation, increases in ventilation concomitant to hypoxia/hypercapnia must be taken into account in studies of cutaneous vascular regulation (8).
With the above information as a background, we performed three separate studies focused on the cutaneous circulation in humans. The purpose of study 1 was to investigate the cutaneous vascular response to hypoxia (in noncral skin) with specific attention to the contribution of sympathetically mediated vasoconstriction to the response. We tested the hypothesis that hypoxia would increase vascular conductance in the skin only when sympathetic vasoconstriction was absent. The purpose of study 2 was to investigate the role of hyperpnea per se in the cutaneous vascular response to hypoxia. We tested the hypothesis that hypoxia, and not hyperpnea, would increase vascular conductance in the skin. The purpose of study 3 was to investigate the cutaneous vascular response to hypercapnia with specific attention to the contribution of sympathetically mediated vasoconstriction to the response. We tested the hypothesis that hypercapnia would increase vascular conductance in the skin, and that the magnitude of vasodilation would be greater when sympathetic vasoconstriction was absent.

METHODS

These studies were approved by the institutional review board of the University of Oregon, and each subject gave written, informed consent before participation.

Subjects. Thirty-five healthy, nonsmoking, normotensive subjects (20 men, 15 women), between the ages of 20 and 33 yr, participated in this series of studies [height 177 ± 10 (SD) cm, weight 74.8 ± 15.3 kg, body mass index 23.8 ± 3.2 kg/m²]. Subjects were taking no medications, except for oral contraceptives, and none had been at altitude (>1,500 m) within 5 mo. Women were studied either during the early follicular phase (1–4 days after the onset of menstruation) of the menstrual cycle or during the placebo phase of oral contraceptive use to minimize the potential effects of female hormones on these responses. All female subjects had a negative urine pregnancy test within 1 h of participation.

Instrumentation. All experiments were performed in thermoneutral conditions with the subject supine, wearing a whole body suit perfused with 33°C water, except during cold stress. When the subject arrived on the study day, two microdialysis fibers (model MD 2000, Bioanalytical Systems, West Lafayette, IN) with a membrane length of 10 mm and molecular mass cutoff of 20 kDa (≤5 μl dead space) were placed in the skin of the ventral forearm. Fibers were placed by inserting a 25-gauge needle through the dermis of the skin in the absence of anesthesia. The fiber was then threaded through the internal lumen of the needle, and the needle was withdrawn, leaving the membrane in place. The fiber was taped in place and perfused with saline solution at a rate of 2 μl/min with a microinfusion pump (Harvard Apparatus, Holliston, MA). Sites were at least 5 cm apart.

After resolution of the microdialysis insertion trauma (~1.5 h), one site was perfused with bretylum tosalate in saline solution (experimental site), while the other site received saline solution only (control). Bretylum was infused for 60 min before initiation of the protocol, and infusions were continuous for the duration of the study (19).

In pilot studies, we attempted to use phentolamine to block α-adrenergic receptors in the skin. This drug was used successfully in our laboratory’s previous investigation of skeletal muscle vascular regulation during hypoxia (41). However, phentolamine produced large variations in resting cutaneous blood flow (unpublished observations). Phentolamine appears to have many vascular effects beyond blocking α-adrenergic receptors, such as blocking K⁺ channels, 5-hydroxytryptamine receptors, and causing histamine release from mast cells (18). Therefore, we used 10 mM bretylum tosalate (Sigma, St. Louis, MO) to block adrenergic neural transmission presynaptically (14). This dose of bretylum has been shown to block adrenergic vasoconstriction in human skin (19, 24, 43).

Measurements. During the drug infusion period, subjects were instrumented for the measurement of heart rate via electrocardiography (Cardiocap/5, Datex-Ohmeda, Madison, WI), ventilation via turbine pneumotach (VMM-400, Interface Associates, Laguna Niguel, CA), arterial pressure via brachial artery auscultation (Cardiocap/5) and photoplethysmography (Finometer, Finapres Medical Systems BV, Arnhem, the Netherlands), arterial O₂ saturation via pulse oximetry (Cardiocap/5), and end-tidal CO₂ via infrared capnography (Cardiocap/5). Isocapnia/eucapnia was defined as the mean end-tidal CO₂ (nasal cannula) during a 5-min period of quiet breathing. To obtain an index of skin blood flow, cutaneous red blood cell flux was measured directly over each microdialysis site by laser-Doppler flowmetry (DRT4, Moor Instruments, Devon, UK) with integrated laser-Doppler probes fixed to the skin with adhesive tape. Skin blood flows were expressed as cutaneous vascular conductance (red blood cell flux/mean arterial pressure) and normalized to baseline values. This method of data presentation is consistent with other investigations focused on cutaneous vascular regulation (1, 17, 24) and has the added benefit of allowing comparison of vascular responses to environmental stress (e.g., hypoxia) across different vascular beds.

To isolate conditions of hypoxia, hyperpnea, and hypercapnia, we used a self-regulating partial-rebreath system developed by Banzett et al. (2) to control alveolar fresh-air ventilation, independent of changes in breathing frequency or tidal volume. Subjects breathed through a scuba mouthpiece with a nose clip to prevent nasal breathing. This system allowed us to clamp end-tidal CO₂ levels, despite large changes in minute ventilation. In our laboratory, the coefficient of variation for end-tidal CO₂ measured during transition from normoxia to hypoxia is 0.02% (n = 5).

Specific protocols. Thirteen subjects participated in study 1. This protocol began with subjects breathing normally on the mouthpiece under normoxic and eucapnic conditions for 5 min (baseline). After this baseline period, the level of O₂ provided in the inspiratory gas was manipulated by mixing normal air and 10% O₂ (balance N₂) via a medical gas blender while controlling alveolar fresh-air ventilation to maintain isocapnia. Subjects were exposed to two levels of isocapnic hypoxia, reducing arterial O₂ saturation to 85 and 80%. Once the target level of hypoxia was reached, it was maintained for 5 min. Hypoxic exposures were separated by 5 min of quiet breathing, during which isocapnia and normoxia were maintained. At the end of the study, the temperature of the water perfusing the whole body suit was decreased to 3–5°C for 5 min to verify adrenergic blockade. All data were collected during the final 2 min of each exposure.

Nine subjects participated in study 2. Again, this protocol began with subjects breathing normally on the mouthpiece under normoxic and eucapnic conditions for 5 min (baseline). After this baseline period, subject’s resting ventilation was determined in terms of tidal volume and respiratory rate. Using these values as a reference, subjects voluntarily increased tidal volume to 300% of the resting value while maintaining respiratory rate. This increase in ventilation was designed to match the hyperpneic strategy used by subjects during hypoxia in study 1. Specific ventilatory strategies were achieved by providing both an auditory and visual cue for breathing frequency and displaying real-time inspiratory tidal volume with predetermined target tidal volumes for each subject. The voluntary hyperpnea lasted 5 min, and normoxia and isocapnia were maintained during this period. Following voluntary hyperpnea, subjects breathed normally on the mouthpiece for 5 min (baseline) before arterial O₂ saturation was reduced to 80%, as described above. Once the target level of hypoxia was reached, it was maintained for 5 min. Hypoxia was followed by another baseline period, during which the subjects breathed normally on the mouthpiece for 5 min. A final voluntary hyperpnea was performed exactly like the first, and the protocol ended with a cold stress, as described in study 1. All data were collected during the final 2 min of each exposure.

Thirteen subjects participated in study 3. Again, this protocol began with subjects breathing normally on the mouthpiece under normoxic
and eucapnic conditions for 5 min (baseline). After this baseline period, subjects’ end-tidal CO2 was increased to 5 and 9 Torr above eucapnic levels by increasing the amount of expired gas in the inspiratory mixture. Once the target level of hypercapnia was reached, it was maintained for 5 min. Hypercapnic exposures were separated by 5 min of normal breathing on the mouthpiece (baseline), and normoxia was maintained throughout by mixing 100% O2 with the inspired gas. After the second hypercapnic exposure, subjects breathed normally on the mouthpiece for 10 min, while hypercapnic ventilation during the second exposure was determined in terms of tidal volume and respiratory rate. Using these values as a target, subjects performed voluntary hyperpnea for 5 min while normoxia and isocapnia were maintained. Target ventilatory parameters were achieved as described above. The protocol ended with a cold stress, as described in study 1, and all data were collected during the final 2 min of each exposure.

Data acquisition and analysis. Data were digitized with signal-processing software (WinDaq, Dataq Instruments, Akron, OH) and analyzed off-line. Results from each study were analyzed with a three-way ANOVA to test the effect of sex on cutaneous vascular responses. There was no effect of sex in any of the studies ($P = 0.995$, $P = 0.749$, and $P = 0.238$ for studies 1–3, respectively). Thus data from men and women were pooled for all subsequent analyses. Pretreatment with bretylium did not significantly affect baseline cutaneous vascular conductance ($P > 0.216$ between pre- and post-bretylium treatment for all studies), as shown previously (19). Therefore, the magnitude of control and bretylium site responses was compared to assess the contribution of sympathetic vasoconstrictor nerves to cutaneous vascular responses. Results were analyzed with a mixed-model ANOVA with SAS (PROC MIXED, SAS version 9.1, SAS Institute, Cary, NC). Differences were considered significant when $P < 0.05$. All values are presented as means ± SE, unless otherwise indicated.

RESULTS

Study 1. Table 1 shows the cardiopulmonary responses to hypoxia in study 1. As planned, arterial O2 saturation decreased ~15 and 20%, with only minimal changes in end-tidal CO2. Tidal volume, ventilation, and heart rate increased during the first ($P = 0.045$, $P = 0.015$, $P < 0.001$) and second (all $P < 0.001$) levels of hypoxia, with increases being greater during the second level ($P < 0.001$ for tidal volume and ventilation; $P = 0.039$ for heart rate). Respiratory rate and mean arterial pressure did not change during either level of hypoxia.

Figure 1 shows cutaneous vascular conductance data from a representative subject in study 1. Data are expressed as a function of time to display the relationship between arterial O2 saturation and cutaneous vascular conductance. Note both the increase in cutaneous vascular conductance as arterial saturation falls and the similarity of the responses in control and bretylium sites. Group responses cannot be displayed as a function of time, because the time required to reach target saturation varied across subjects. Figure 2 shows group cutaneous vascular conductance responses to hypoxia in study 1. Upon reduction of arterial O2 saturation to 85%, cutaneous vascular conductance increased to 120 ± 10% baseline in the control site ($P = 0.011$), with a trend to increase in the bretylium site (113 ± 3% baseline; $P = 0.087$). Upon reduction of arterial O2 saturation to 80%, cutaneous vascular conductance increased to 126 ± 7% of baseline in the control site ($P < 0.001$) and 121 ± 4% baseline in the bretylium site ($P < 0.001$). Responses were not different between control and bretylium sites at either level of hypoxia (both $P > 0.453$). In addition, responses were not different between hypoxic levels in either site (both $P > 0.442$). Cold stress caused a reduction in cutaneous vascular conductance to 56 ± 5% baseline in the control site ($P < 0.001$), while the bretylium site remained unchanged (97 ± 3% baseline; $P = 0.417$).

Study 2. Table 2 shows the cardiopulmonary responses to hyperpnea and hypoxia in study 2. Hypoxia, but not hyperpnea, caused a decrease in arterial O2 saturation ($P < 0.001$). While neither end-tidal CO2 nor respiratory rate changed throughout the protocol, tidal volume and ventilation increased with hyperpnea in study 2.

<table>
<thead>
<tr>
<th>Table 1. Cardiopulmonary responses to hypoxia in study 1</th>
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<tbody>
<tr>
<td><strong>Arterial O2 saturation, %</strong></td>
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<tr>
<td>-----------------------------</td>
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<tr>
<td>98.4 ±0.2</td>
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<tr>
<td>End-tidal CO2, Torr</td>
</tr>
<tr>
<td>Ventilation, 1/min</td>
</tr>
<tr>
<td>Tidal volume, liters</td>
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<tr>
<td>Respiratory rate, breaths/min</td>
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<tr>
<td>Heart rate, beats/min</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
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</tbody>
</table>

Values are means ± SE; $n = 13$. *$P < 0.05$ vs. baseline. †$P < 0.05$ between hypoxic exposures.

Fig. 1. Representative responses in cutaneous vascular conductance (CVC), normalized to baseline, to two levels of hypoxia in a subject in study 1. One site was treated with bretylium tosylate (●), and the other received saline as a control (○). Note the increases in CVC as arterial O2 saturation (SaO2) falls and the similarity in the bretylium and control responses.

Fig. 2. Group responses in CVC, normalized to baseline, to two levels of hypoxia in study 1. One site was treated with bretylium tosylate, and the other received saline as a control. Values are means ± SE; $n = 13$. *$P < 0.05$ for normalized CVC vs. preexposure baseline.
hypercapnia and hyperpnea during unchanged (96/11006 caused no change in end-tidal CO2, heart rate, or mean arterial pressure increased only during the more severe hyperpnea. The rate and mean arterial pressure increased with hypoxia only (P = 0.004 and P = 0.016).

Figure 3 shows group cutaneous vascular responses to hyperpnea and hypoxia in study 2. Before hypoxia, hyperpnea caused no change in cutaneous vascular conductance in the control site (101 ± 4% baseline; P = 0.839) or bretylium site (97 ± 2% baseline; P = 0.372). During hypoxia, cutaneous vascular conductance increased to 115 ± 7% baseline in the control site (P = 0.027) and 118 ± 4% baseline in the bretylium site (P = 0.012). After hypoxia, hyperpnea again caused no change in cutaneous vascular conductance in the control site (102 ± 3% baseline; P = 0.622) or bretylium site (99 ± 3% baseline; P = 0.849). Responses were not different between control and bretylium sites during hypoxia or hyperpnea (all P > 0.440). Cold stress caused a reduction in cutaneous vascular conductance to 58 ± 7% baseline in the control site (P < 0.001), while the bretylium site remained unchanged (96 ± 3% baseline; P = 0.410).

Study 3. Table 3 shows the cardiopulmonary responses to hypercapnia and hyperpnea during study 3. While arterial O2 saturation did not change throughout the protocol, hypercapnia caused graded increases in end-tidal CO2, tidal volume, ventilation, and respiratory rate (all P < 0.001 vs. baseline; P < 0.001 between hypercapnic exposures). Heart rate and mean arterial pressure increased only during the more severe hypercapnic exposure (P < 0.001 and P = 0.005). Hyperpnea alone caused no change in end-tidal CO2, heart rate, or mean arterial pressure (all P > 0.627). As planned, hyperpnea increased tidal volume, ventilation, and respiratory rate equal to levels induced by the more severe hypercapnic exposure (all P > 0.645 between hypercapnia and hyperpnea).

Figure 4 shows group cutaneous vascular conductance responses to hypercapnia and hyperpnea in study 3. During the first level of hypercapnia (+5 Torr), cutaneous vascular conductance increased to 111 ± 4% baseline in the control site (P = 0.004), but no change was observed in the bretylium site (102 ± 3% baseline; P = 0.508). During the second level of hypercapnia (+9 Torr), cutaneous vascular conductance increased to 111 ± 4% baseline in the control site (P = 0.024), but no change was observed in the bretylium site (106 ± 4% baseline; P = 0.182). Voluntary hyperpnea caused no change in cutaneous vascular conductance in the control site (99 ± 7% baseline; P = 0.821) or bretylium site (98 ± 4% baseline; P = 0.719). The increase in cutaneous vascular conductance during the first level of hypercapnia was greater in the control site than in the bretylium site (P = 0.048 between sites). Responses were not different between control and bretylium sites during the second level of hypercapnia or hyperpnea (both P > 0.369). In addition, responses were not different between hypercapnic levels in either site (both P > 0.494). Cold stress caused a reduction in cutaneous vascular conductance to 60 ± 5% baseline in the control site (P < 0.001), while the bretylium site remained unchanged (98 ± 5% baseline; P = 0.550).

DISCUSSION

The primary finding of this series of studies is that hypoxia causes modest but consistent vasodilation in nonacral skin that is not affected by sympathetically mediated vasoconstriction. Hypercapnia also causes cutaneous vasodilation, and sympathetically mediated vasoconstriction may be important in this response. Neither response is reproduced by matched hyperpnea.

Traditionally, the peripheral vascular responses during hypoxia and hypercapnia have been viewed as a competition between chemoreflex control, mediated primarily through the sympathetic nervous system, and local vasodilator influences (16, 33). This model implies that sympathetic outflow is activated diffusely to peripheral vascular beds during chemoreceptor stimulation. Therefore, our hypotheses regarding the effect of bretylium pretreatment on hypoxic and hypercapnic vasodilation were based on the assumption that the cutaneous vasculature would receive increased sympathetic outflow during chemoreceptor stimulation, primarily in the form of vasoconstrictor nerve activity. However, many studies suggest that sympathetic activation during chemoreceptor stimulation is end-organ specific (13, 20, 21, 38). Therefore, this model of regulatory control may not hold true for the entire peripheral

Table 2. Cardiopulmonary responses to hyperpnea and hypoxia in study 2

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Hyperpnea</th>
<th>Hypoxia (80%)</th>
<th>Hyperpnea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial O2 saturation, %</td>
<td>98.7 ± 0.2</td>
<td>99.0 ± 0.2</td>
<td>79.8 ± 0.3*</td>
<td>99.0 ± 0.2</td>
</tr>
<tr>
<td>End-tidal CO2, Torr</td>
<td>39.8 ± 0.8</td>
<td>40.0 ± 0.7</td>
<td>39.8 ± 0.7</td>
<td>39.9 ± 0.7</td>
</tr>
<tr>
<td>Ventilation, l/min</td>
<td>6.1 ± 0.6</td>
<td>19.0 ± 1.7*</td>
<td>13.3 ± 1.4†</td>
<td>20.0 ± 1.9*</td>
</tr>
<tr>
<td>Tidal volume, liters</td>
<td>0.5 ± 0.0</td>
<td>1.5 ± 0.2*</td>
<td>1.0 ± 0.1†</td>
<td>1.6 ± 0.2*</td>
</tr>
<tr>
<td>Respiratory rate, breaths/min</td>
<td>13.1 ± 0.8</td>
<td>12.7 ± 0.9</td>
<td>13.9 ± 1.4</td>
<td>12.8 ± 0.9</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>50.2 ± 3.6</td>
<td>58.3 ± 5.4</td>
<td>72.4 ± 5.0*</td>
<td>62.2 ± 3.4</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>89.7 ± 1.5</td>
<td>91.8 ± 1.9</td>
<td>92.8 ± 2.1*</td>
<td>94.0 ± 2.3</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 9. *P < 0.05 vs. baseline; †P < 0.05 between hypoxia and hyperpnea.
Table 3. Cardiopulmonary responses to hypercapnia and hyperpnea in study 3

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Hypercapnia (+5 Torr)</th>
<th>Hypercapnia (+9 Torr)</th>
<th>Hyperpnea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial O₂ saturation, %</td>
<td>99.3 ± 0.1</td>
<td>99.5 ± 0.1</td>
<td>99.4 ± 0.1</td>
<td>99.4 ± 0.1</td>
</tr>
<tr>
<td>End-tidal CO₂, Torr</td>
<td>40.6 ± 0.7</td>
<td>46.5 ± 0.7 *</td>
<td>50.7 ± 0.8 †</td>
<td>40.6 ± 0.9</td>
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<tr>
<td>Ventilation, l/min</td>
<td>7.1 ± 0.7</td>
<td>18.4 ± 2.2 *</td>
<td>33.2 ± 2.9 †</td>
<td>33.1 ± 2.6*</td>
</tr>
<tr>
<td>Tidal volume, liters</td>
<td>0.7 ± 0.1</td>
<td>1.5 ± 0.3 *</td>
<td>2.1 ± 0.3 †</td>
<td>2.2 ± 0.3*</td>
</tr>
<tr>
<td>Respiratory rate, breaths/min</td>
<td>11.3 ± 1.0</td>
<td>14.4 ± 1.5 *</td>
<td>17.2 ± 1.3 †</td>
<td>16.8 ± 1.4*</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>54.2 ± 2.8</td>
<td>57.4 ± 2.7</td>
<td>62.8 ± 3.4 †</td>
<td>56.1 ± 3.5</td>
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<tr>
<td>Mean arterial pressure, mmHg</td>
<td>87.8 ± 1.8</td>
<td>90.5 ± 2.0</td>
<td>94.8 ± 2.7 †</td>
<td>90.7 ± 2.4</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 13. *P < 0.05 vs. baseline. †P < 0.05 between hypercapnic exposures.

vasculature (e.g., the skin). We are unaware of any microneurographic recordings of skin sympathetic nerve activity directed to nonacral skin in humans exposed to hypoxia. Furthermore, any such recordings would be difficult to interpret because of the dual innervation of nonacral skin in humans (see Limitations).

In skeletal muscle, the net response to hypoxia is vasodilation, despite reflex increases in muscle sympathetic vasoconstrictor nerve activity (35, 36). We have shown that, when α-receptor-mediated vasoconstriction is blocked with brachial artery infusion of phentolamine, a larger vasodilation is observed, indicating that adrenergic vasoconstriction “masks” a portion of the hypoxic vasodilation in skeletal muscle (41). In that same study, we collected skin blood flow data in a subset of subjects to address the possibility that cutaneous vasodilation might also be restrained by adrenergic vasoconstriction. Although our initial findings in that study suggested the presence of overlying α-adrenergic vasoconstriction, the current findings indicate that cutaneous vasodilation is not masked by sympathetically mediated vasoconstriction during hypoxia. The main evidence for this is that skin blood flow increased the same amount during hypoxia in the control skin site and the site with sympathetic vasoconstrictor blockade. The apparent disagreement between our present findings and those published previously could be explained by methodological differences. First, our previous experimental design was focused on skeletal muscle vascular regulation, and, as such, cutaneous vascular responses to hypoxia were collected in only six subjects. In light of the modest magnitude of hypoxic responses in skin, we may not have studied enough subjects to detect a vasodilation in the control site (power analysis based on the average variance in the two studies predicts that power is 0.399 for 6 subjects, but 0.811 for 13 subjects). Thus a type II statistical error may have been made. Along these lines, the P value for the control response during hypoxia in our previous study was P = 0.10 (i.e., there was a trend toward vasodilation, and the magnitude of that trend was similar to that in the present study), but vasodilation was not compared between control and α-adrenergic blocked sites. Therefore, the basis for the suggestion that vasoconstriction might mask hypoxic vasodilation in the skin (the fact that the increase in skin blood flow failed to reach significance in the control site) may have disappeared if a few more subjects had been studied. The present investigation more than doubled the previous subject sample in study 1 (n = 13 vs. 6). Moreover, a separate group of subjects (n = 9) was exposed to hypoxia in study 2, and the cutaneous vasodilation was not affected by sympathetic vasoconstrictor blockade in that study either. In both studies 1 and 2, modest but consistent increases in cutaneous vascular conductance were seen during hypoxia. These results indicate that sympathetically mediated vasoconstriction does not mask cutaneous vasodilation during hypoxia, and that hypoxia causes modest but consistent vasodilation in nonacral skin.

A second methodological consideration that may account for differences in our current and previous findings is the use of bretylium tosylate in the current investigation, instead of phentolamine (previous study). When we attempted to use phentolamine to block cutaneous α-adrenergic receptors in the present investigation, large variations in resting cutaneous blood flow were observed (unpublished observations). Phentolamine appears to have many vascular effects beyond blocking α-adrenergic receptors, such as blocking K⁺ channels and 5-hydroxytryptamine receptors, and causing histamine release from mast cells (18). Our initial attempts in which the skin was perfused with phentolamine via microdialysis would suggest that some of these effects could be present in the cutaneous circulation. Thus it is possible that phentolamine had a nonspecific effect on the cutaneous vasculature in our previous investigation, causing a progressive elevation in resting skin blood flow that was superimposed on hypoxic vasodilation. This would explain why an increase in cutaneous vascular conductance was detected in the phentolamine-treated arm, and not the control arm, during hypoxia.

An alternative interpretation of the data collected with bretylium and phentolamine may provide insight into the origin of a cutaneous vasoconstrictor stimulus during hypoxia. The discrepant findings regarding adrenergic vasoconstriction in the skin during hypoxia may be explained by the different locations at which bretylium and phentolamine interfere with

Fig. 4. Group responses in CVC, normalized to baseline, to two levels of hypercapnia, followed by matched hyperpnea, in study 3. One site was treated with bretylium tosylate, and the other received saline as a control. Values are means ± SE; n = 13. *P < 0.05 for normalized CVC vs. preexposure baseline. †P < 0.05 vs. control site within exposure.
vasoconstriction. For instance, phentolamine blocks postsynaptic α1- and α2-adrenergic receptors (18), and bretylum tosylate blocks adrenergic nerve transmission presynaptically (14). Blockade of α1- and α2-adrenergic receptors interferes with both humoral and neural adrenergic vasoconstriction, whereas only neural vasoconstriction is blocked by inhibition of sympathetic adrenergic fibers. Thus our previous finding of vasodilation during α-receptor blockade only may indicate that humoral vasoconstriction is present in the skin during hypoxia. This vasoconstriction would have been blocked by phentolamine in the previous study, but not by bretylum in the present study. Therefore, no differences between control and drug sites would be expected in the present study. Along these lines, hypoxia caused an elevation in circulating epinephrine in our laboratory’s previous investigation (41). Epinephrine has been shown to cause cutaneous vasoconstriction (humoral) when administered intra-arterially, intravenously, by subcutaneous injection, or by iontophoresis (3, 5, 18, 31). Taken together, these observations suggest that adrenergic vasoconstriction of humoral origin (possibly epinephrine) may mask some of the cutaneous vasodilation during hypoxia, and adrenergic vasoconstriction of neural origin (i.e., sympathetic vasoconstrictor nerves) does not affect hypoxic vasodilation in skin.

In light of the suggestion that epinephrine could cause humoral vasoconstriction in the skin during hypoxia, it should be noted that β-adrenergic receptors have been identified in the skin using isoproterenol (6). However, isoproterenol has a greater affinity for β-receptors than epinephrine, and almost no affinity for α-receptors (12, 18). Therefore, it may be possible to stimulate β-receptors pharmacologically in the skin, while the physiological response to epinephrine is dictated by the rich distribution of vascular α-receptors (18). An additional point regarding the effects of bretylum and phentolamine on hypoxic vasodilation is that, as mentioned above, phentolamine was infused into the brachial artery in our laboratory’s previous investigation (41), whereas intradermal microdialysis was used to deliver bretylum in the present studies. It has been demonstrated that brachial artery infusions of vasoactive agents do not consistently produce desired concentrations in the cutaneous vasculature (37). We have no way of knowing if this occurred in our previous investigation. However, in the current context, any failure of phentolamine to reach target concentrations in the cutaneous circulation in our previous study would have resulted in underestimation of adrenergic vasoconstriction during hypoxia, further strengthening the case for humorally mediated vasoconstriction in the skin.

To our knowledge, the mechanisms underlying hypoxic vasodilation in nonacral skin are unknown. The nonacral cutaneous vasculature is innervated by two branches of the sympathetic nervous system: an adrenergic vasoconstrictor system and a cholinergic vasodilator system (22, 24, 25, 31, 32). While our data show that the adrenergic vasoconstrictor system is not involved in cutaneous vasodilation during hypoxia, the active vasodilator system may play a role. Along these lines, our laboratory has previously shown that blockade of nitric oxide production in the forearm reduces hypoxic vasodilation in the skin (41). These data suggest that nitric oxide plays a role in cutaneous vasodilation during hypoxia. Nitric oxide contributes to cutaneous vasodilation during thermal stress via direct actions on vascular smooth muscle and synergistic activity with an unknown neurotransmitter associated with the active vasodilator system (42). Thus the nitric oxide dependence of cutaneous vasodilation during hypoxia may indicate a role for active vasodilation. Conversely, nitric oxide may represent a pathway through which local vasodilator mechanisms act, as has been suggested in skeletal muscle during hypoxia (4, 15). At present, we are unable to determine whether cutaneous vasodilation during hypoxia occurs through local or neural vasodilator mechanisms.

In study 3, cutaneous vasodilation during hypercapnia was only observed when adrenergic vasoconstriction was intact. These data suggest the possibility that cutaneous vasodilation occurs through withdrawal of vasconstrictor tone during hypercapnia. This mechanism is supported by the previous work of Gregor and Jänig (13), in which direct recordings from cutaneous vasoconstrictor neurons were obtained in the cat. In that study, exposure to hypercapnia caused a reduction in vasconstrictor neural impulses directed to the skin. An additional possibility is that elevated CO2 tension in the cutaneous tissue interferes with transduction of vasoconstriction, either by decreasing the responsiveness of α-receptors on the vasculature or by altering norepinephrine release from adrenergic nerve terminals. This blunted transduction could occur in response to CO2 per se, or acidosis secondary to elevated CO2. Along these lines, McGillivray-Anderson and Faber (27) demonstrated a reduction in α2-receptor-mediated vasoconstriction under acidic conditions produced by adding CO2 to a cremaster preparation. These results suggest a role for CO2 in blocking vasconstrictor transduction, possibly through acidosis. While no single mechanism can be assigned at this point, our results show that hypercapnia causes modest but consistent vasodilation in nonacral skin that is abolished with sympathetic vasconstrictor blockade. The magnitude of hypercapnic vasodilation (~10%) supports the mechanisms proposed above insofar as basal cutaneous vasconstrictor tone should have been minimal with whole body skin temperature clamped at 33°C (33).

Comparisons of response magnitude between intact and sympathetic vasconstrictor blocked sites only support the above mechanisms during the first level of hypercapnia. That is, during more severe hypercapnia, there was no difference in the way that intact and adrenergic-blocked sites responded, even though vasodilation was only detected in the intact site. The reason for this is unclear. It may be that there is a local vasodilation of cutaneous vessels to CO2, but we did not use a large enough hypercapnic stimulus to observe this effect. If there is a local effect of CO2, as suggested by earlier studies (10, 39), subtle effects at concentrations below the threshold for vasodilation would obscure differences between intact and α-adrenergic blocked sites to a greater degree closer to that threshold (i.e., at relatively higher concentrations). This could explain the lack of difference between intact and adrenergic blocked sites during the more severe level of hypercapnia.

Direct recordings of cutaneous sympathetic nerve activity show that hyperventilation causes elevated sympathetic outflow to the skin (8). Therefore, subjects who participated in studies 2 and 3 performed voluntary isocapnic (normoxic) hyperpnea to address the role of hyperpnea per se in the cutaneous vascular responses to hypoxia and hypercapnia. Ventilatory strategies were adjusted to specifically match those employed during either hypoxia (study 2) or hypercapnia (study 3). Subjects in study 2 performed hyperpnea both before
and after hypoxic exposure to control for lasting changes in sympathetic neural outflow after hypoxia (29, 40). Throughout these studies, hyperpnea failed to reproduce the hypoxic or hypercapnic responses in the cutaneous circulation. Therefore, the cutaneous vascular responses during hypoxia and hypercapnia were not an effect of hyperpnea per se.

Limitations. One limitation of our series of studies is that skin sympathetic nerve activity was not recorded. While this does not limit our ability to describe vascular changes during hypoxia and hypercapnia, it could limit our interpretation of the mechanisms underlying these vascular changes (i.e., sympathetic vasoconstrictor withdrawal vs. blunted transduction). However, measurement of skin sympathetic nerve activity in humans is incapable of distinguishing the dual neural input to the cutaneous vasculature (i.e., adrenergic vasoconstrictor and active vasodilator nerves). Furthermore, vascular changes are not always reflected by changes in skin sympathetic nerve activity, indicating that this may be a poor index of autonomic control directed to the skin (7).

Perspectives. The cutaneous vascular responses observed during hypoxia and hypercapnia are modest in magnitude compared with thermoregulatory vasodilation (44). However, these modest changes in perfusion may impact thermoregulation in important ways. For example, exposure to acute isocapnic hypoxia increases the rate of core cooling in cold-stressed humans (23). Such acceleration in heat loss is consistent with elevated skin blood flow and increased core-to-skin heat transfer. However, simultaneous measurements of nonacral skin blood flow and core temperature during exposure to hypoxia and cold stress have not been made. Thus a causal link between accelerated core cooling and increased cutaneous perfusion during hypoxia has yet to be established.

Conclusions. In summary, this series of studies provides evidence that both hypoxia and hypercapnia cause modest vasodilation in nonacral skin. Adrenergic vasoconstriction of neural origin does not restrain hypoxic vasodilation, but may be important in hypercapnic vasodilation. The hypercapnea associated with hypoxia and hypercapnia does not mediate the vascular responses in the skin.

ACKNOWLEDGMENTS

The authors express gratitude to all of the subjects who participated in this series of studies. The authors especially thank Dr. Brad Wilkins and Mollie Pricher for invaluable assistance in developing these studies and Robin High for statistical expertise. These studies were conducted by G. H. Simmons in partial fulfillment for the degree of Masters of Science in the Department of Human Physiology at the University of Oregon.

GRANTS

This research was supported by a grant from the American Heart Association, Northland Affiliate, Scientist Development Grant 30403Z, and National Heart, Lung, and Blood Institute Grants HL-65305 (J. R. Halliwill) and HL-90297 (C. T. Minson). J.-L. Cracowski was supported by grants from AGIR à Dom and Association Nationale pour les Traitements à Domicile, les Innovations et la Recherche.

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