Evidence of sustained forearm vasodilatation after brief isocapnic hypoxia

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Tamisier, Renaud, Daniel Norman, Amit Anand, Yoon Choi, and J. Woodrow Weiss. Evidence of sustained forearm vasodilatation after brief isocapnic hypoxia. J Appl Physiol 96: 1782–1787, 2004.—Healthy subjects exposed to 20 min of hypoxia increased vasodilation and muscle sympathetic nerve activity (MSNA). After return to normoxia, although ventilation returns to baseline, MSNA remains elevated for up to an hour. Because forearm vascular resistance is not elevated after hypoxic exposure, we speculated that the increased MSNA might be a compensatory response to sustained release of endogenous vasodilators. We studied the effect of isocapnic hypoxia (mean arterial oxygen saturation 81.6 ± 4.1%, end-tidal PCO2 44.7 ± 6.3 Torr) on plethysmographic forearm blood flow (FBF) in eight healthy volunteers while infusing intra-arterial phentolamine to block local α-receptors. The dominant arm served as control. Forearm arterial vascular resistance (FVR) was calculated as the mean arterial pressure (MAP)-to-FBF ratio. MAP, heart rate (HR), and FVR were reported at 5-min intervals at baseline, then while infusing phentolamine throughout the study. By design, FVR decreased during phentolamine infusion. Hypoxia further decreased FVR in both forearms. With continued phentolamine infusion, FVR after termination of the exposure (17.47 ± 8.51 mmHg·min·ml−1·100 ml of tissue) remained lower than preexposure baseline value (23.05 ± 8.51 mmHg·min·ml−1·100 ml of tissue; P < 0.05). We conclude that, unmasked by phentolamine, the vasodilatation occurring during hypoxia persists for at least 30 min after the stimulus. This vasodilatation may contribute to the sustained MSNA rise observed after hypoxia.

METHODS

Healthy volunteers served as subjects. All were free of cardiovascular, pulmonary, endocrine, and neurological diseases as evaluated by history and physical examination. All subjects were nonsmokers who were taking no medications except oral contraceptives. Women were studied during the first week of their menstrual cycle. The protocol was reviewed and approved by the Committee on Clinical Investigations, the appropriately authorized Institutional Review Board for research involving human subjects at the Beth Israel Deaconess Medical Center. All subjects provided written, informed consent.

General procedures. Subjects were instrumented in the supine position. Respiratory and cardiovascular variables were digitized (model DL-720 series, DatQ Instruments, Akron, OH) and recorded continuously on a computer for offline data analysis (Windaq, DatQ Instruments).

After local anesthesia (2–3 ml of 1% lidocaine), a 5-cm, 20-gauge catheter was placed in the brachial artery of the nondominant arm (left in all cases) under sterile conditions. We measured intra-arterial pressure and infused phentolamine through this catheter by using a three-way stopcock (Baxter, Deerfield, IL) placed in series with the catheter-transducer system (Transpac II, Abbott Critical Care Systems, Chicago, IL). The catheter was continuously flushed (3 ml/h) with heparinized (2 U/ml) saline.

Subjects breathed through a leak-free face mask (8940 series, Hans Rudolph, Kansas City, MO) to which a two-way valve was connected (2600 series, Hans Rudolph). A pneumotachograph (Hewlett-Packard, Palo Alto, CA) was attached to the inspiratory tubing for measurement of respiratory flow and rate. Tidal volume was calculated by integration of flow. Minute ventilation was calculated by summing tidal volume of each period selected and dividing by time. End-tidal carbon dioxide concentrations were continuously monitored from the expiratory limb of the pneumotachograph using an infrared analyzer (model 4201, DatQ Instruments). 

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dioxide tension (PETCO₂) was measured by gas analyzer (model 17630, Vacu-Med, Ventura, CA). Arterial oxygen saturation (SaO₂) was measured by using a pulse oximeter (Biox model 3740, Ohmeda, Louisville, CO). Heart rate was calculated from the mean R-R interval on the electrocardiogram (model M-90, Medical Research Laboratories, Buffalo Grove, IL). Blood flow was measured in both forearms by venous occlusion plethysmography (model EC6 plethysmograph, Hokanson, Bellevue, WA). During this measurement, forearms were elevated above the level of the right atrium to collapse the veins. The arterial occlusion wrist cuffs were inflated to 200 mmHg (or 50 mmHg above the highest resting arterial pressure). Then, when the signal stabilized (after 45–60 s), a collecting cuff positioned above the elbow was rapidly inflated to 50 mmHg for 8 s every 16 s. The average of four to six flow measurements was used as each data point in the computation of the results.

Vascular resistance (in mmHg·min·m⁻¹·100 ml of tissue) for each forearm was calculated as the ratio between mean intra-arterial pressure and forearm blood flow (in ml·100 ml of tissue⁻¹·min⁻¹).

Experimental protocols. After 30 min of baseline recording, a 5-min loading dose (100 μg/min) of phentolamine was begun and then followed by a continuous infusion (25 μg/min) until the end of the study (Figs. 1 and 2). After 60 min of recordings on room air (30 min without and 30 min with phentolamine), we started the exposure to isocapnic hypoxia. A gas mixture with 9% oxygen, balance nitrogen, was added to the breathing circuit. Carbon dioxide and nitrogen were added to the inspiratory gas mixture as needed to reach a SaO₂ of 80% and PETCO₂ equivalent to preexposure levels. The 20 min of exposure were considered to start once the target levels of SaO₂ and PETCO₂ were reached and were followed by a 30-min recovery period on room air. Heart rate and ventilation variables were continuously recorded from 15 min before through 15 min after the exposure and were analyzed in 5-min segments. Blood pressure and vascular flow were recorded during 90- to 120-s periods every 5 min.

Data analysis. Ten healthy volunteers were enrolled in the study, but only eight are included in the analysis because we were unsuccessful in placing the arterial line in two of the subjects. To characterize the chemical stimuli and the ventilatory responses associated with hypoxia, average values for PETCO₂, SaO₂, and minute ventilation were computed for the baseline, exposure, and recovery periods. Statistical analyses were performed by using SPSS software (SPSS, Chicago, IL). Data on room air for 30 min before and then 30 min after phentolamine infusion were averaged as each of two baseline values; then subsequent data from the start of hypoxic exposure through the end of the recovery period were analyzed in 5-min intervals. All 5-min periods were compared with baseline, and experimental and control arms were compared by a general linear model with repeated measures. Except where otherwise noted, data are reported as means ± SD in the text, tables, and figures. Values of \( P < 0.05 \) were considered statistically significant.

RESULTS

Subjects. Eight healthy volunteers (ages 28.13 ± 8.98 yr, 1 woman) were included in the study. Subjects’ anthropometrical data are displayed in Table 1. Figure 3 has sample tracings from baseline, during isocapnic hypoxia exposure, and recovery periods, showing representative recordings of arterial pressure, forearm blood flow, SaO₂, and respiratory flow.

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age, yr</th>
<th>Weight, kg</th>
<th>Height, m</th>
<th>BMI, kg/m²</th>
<th>Gender</th>
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<tbody>
<tr>
<td>1</td>
<td>48</td>
<td>90.8</td>
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<td>28.72</td>
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<tr>
<td>2</td>
<td>25</td>
<td>100.0</td>
<td>1.83</td>
<td>29.86</td>
<td>M</td>
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<tr>
<td>3</td>
<td>29</td>
<td>65.5</td>
<td>1.79</td>
<td>20.42</td>
<td>M</td>
</tr>
<tr>
<td>4</td>
<td>33</td>
<td>75.0</td>
<td>1.79</td>
<td>23.40</td>
<td>M</td>
</tr>
<tr>
<td>5</td>
<td>22</td>
<td>59.0</td>
<td>1.75</td>
<td>19.26</td>
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</tr>
<tr>
<td>6</td>
<td>21</td>
<td>72.5</td>
<td>1.80</td>
<td>22.33</td>
<td>M</td>
</tr>
<tr>
<td>7</td>
<td>22</td>
<td>59.0</td>
<td>1.68</td>
<td>20.97</td>
<td>F</td>
</tr>
<tr>
<td>8</td>
<td>25</td>
<td>79.0</td>
<td>1.81</td>
<td>24.11</td>
<td>M</td>
</tr>
</tbody>
</table>

Mean ± SD 28.13 ± 8.98 75.10 ± 14.63 1.78 ± 0.05 23.63 ± 3.84 7/1 (M/F)

BMI, body mass index; M, male; F, female.
Hypoxic exposure and ventilatory response. Mean hemoglobin \(\text{SaO}_2\) was 98.22 ± 1.32% at baseline, decreased to 81.63% ± 4.12% \((P < 0.001)\) during exposure, and then returned to 98.83 ± 0.85% in the recovery period. \(\text{PaCO}_2\) went from 43.09 ± 5.65 Torr at baseline, to 44.73 ± 6.27 Torr during exposure, and to 41.18 ± 1.54 Torr after 5 min of recovery, not a statistically significant change. Minute ventilation increased significantly during hypoxia and returned to baseline during the recovery period (Table 2). Compared with baseline, respiratory rate increased during hypoxia, reaching statistical significance only 15 min into the hypoxic exposure (from 12.30 ± 4.06 to 14.79 ± 3.70 breaths/min; \(P < 0.05\)). Tidal volume showed a similar trend, but the absolute increase in tidal volume vs. baseline was only significant for the first 5 min of exposure (from 0.59 ± 0.26 to 0.80 ± 0.32 liter; \(P < 0.05\)). Both respiratory rate and tidal volume returned to baseline values during the recovery period.

Effects of hypoxic exposure on hemodynamic response. Mean arterial pressure (Fig. 4) increased significantly from baseline to hypoxia \((P < 0.001)\), but (as predicted) there was no change in vascular resistance in the control arm. During exposure to hypoxia (Fig. 5), the control forearm vascular resistance trended downward by 11–14%, representing a significant change from baseline during the last 10 min of the exposure \((P < 0.05)\). In the experimental forearm, the hypoxic exposure caused vascular resistance to decline an additional 40% compared with phentolamine infusion, and 63% compared with preinfusion room air values \((P < 0.001)\). In the recovery period, control arm forearm vascular

Table 2. Effects of isocapnic hypoxia on ventilation

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Hypoxia</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 min</td>
<td>10 min</td>
<td>15 min</td>
</tr>
<tr>
<td>Respiratory rate, breaths/min</td>
<td>12.30±4.06</td>
<td>13.31±3.89</td>
<td>13.86±3.67</td>
</tr>
<tr>
<td>Tidal volume, liter</td>
<td>0.59±0.26</td>
<td>0.80±0.29*</td>
<td>0.80±0.28</td>
</tr>
<tr>
<td>Minute ventilation, l/min</td>
<td>6.37±0.77</td>
<td>9.93±3.73*</td>
<td>10.64±4.20*</td>
</tr>
</tbody>
</table>

Values are means ± 5D. Ventilation values are reported as the mean values for the entire periods of baseline and for each 5-min interval during the 20-min exposure or the first 15 min of recovery. Statistical analysis was figured with \(*P < 0.05\) and \(†P < 0.01\) when analyzed compared with baseline (preexposure values).
apparently due to increased sympathetic activity. In the control limb, this vasodilation was obscured, -adrenergic blockade with phentolamine, forearm vasodilation was evident after induction of a similar period of hypercapnic hypoxia. The increase in sympathetic activity was not evident during an exposure to poikilocapnic hypoxia. Although they demonstrated that aminophylline reduces the vasodilatory response to hypoxia, the interpretation of these data is complicated by the fact that aminophylline itself causes substantial vasodilatation, which may limit the degree to which hypoxia could further dilate the local arterial bed (14). Epinephrine may also affect vascular resistance, as a humoral factor that can cause substantial β-adrenergic-receptor-mediated vasodilation. Using an intra-arterial infusion of propanolol, Weisbrod et al. (18) demonstrated that local β-blockade reduced by 50% the vasodilation observed during hypoxic exposure. Because nitric oxide (NO) is known to drive hypoxic vasodilation (2, 3), Weisbrod et al. completed their experiment with blockade of NO synthase, the enzyme responsible for production of NO. This had an effect on basal vascular tone in that study (vasoconstriction), but it had no additional effect on the vascular response to hypoxia beyond that produced by propanolol. This suggests that epinephrine-mediated vasodilation to hypoxia may ultimately act on the NO pathway through activation of β-receptors. Finally, there is also evidence that newly synthet-
sized prostaglandins (PG) may be involved in hypoxia-induced vasodilation (16). Moreover, the interrelationships between NO, adenosine, and PG have been investigated during hypoxia in animals. Ray et al. (16) demonstrated that, during hypoxia, adenosine is involved in the endothelial release of both NO and PG and that NO release was dependent on PG synthesis (16).

Although most investigators have focused on the release of endogenous vasodilators to account for forearm vasodilation during hypoxia, another possibility is that decreased levels of a vasoconstrictor mechanism or a decrease in postjunctional \( \alpha \)-adrenergic effects might contribute to the decrease in vascular tone during hypoxic exposure. There is evidence that norepinephrine clearance is increased during exposure to hypoxia (13), but the functional consequence of this augmented clearance is unclear. Postjunctional \( \alpha \)-adrenergic-receptor vasoconstrictor responsiveness to endogenous norepinephrine release is not blunted during systemic hypoxia (5). Other compounds, such as endothelin, that contribute to resting vascular tone may increase with prolonged exposure to hypoxia, but changes in endothelin during brief hypoxia have not been identified.

Although our data support persistent vasodilation after exposure to hypoxia, several limitations of our study should be noted. First, we studied only isocapnic hypoxia. Several studies have demonstrated sustained sympathoexcitation after isocapnic hypoxia and hypercapnic hypoxia but not after hypercapnic hyperoxia (15, 20). Thus it seems likely that hypoxia is necessary, but it is still unclear whether hypoxia is sufficient for these findings to occur. That is, we do not know whether poikilocapnic hypoxia would also result in sustained vasodilation. Nor do we know the severity of hypoxia required to produce these findings. Our subjects experienced moderate hypoxemia, with \( \text{SaO}_2 \) falling to \( \sim 80\% \) during the exposure. We did not assess less severe hypoxia to determine whether a milder challenge would produce similar results.

Second, we did not measure MSNA in this study. We believe that the sustained MSNA response to hypoxic exposure has been extensively described by others (15, 20) and in our laboratory (R. Tamisier, L. Nieto, A. Anand, D. Cunnington, and J. W. Weiss, unpublished data). Also, it is well accepted that the local arterial infusion of phentolamine protocol that we followed specifically targets sympathetic vascular tone (18).

Third, we did not confirm the adequacy of our \( \alpha \)-adrenergic blockade. However, the phentolamine dosage (100 \( \mu \)g/min loading dose and then 25 \( \mu \)g/min) we used during this study was the same as that used by other authors (18) who have performed dose-response testing to validate that these doses produce complete \( \alpha \)-adrenergic blockade. To achieve a stable baseline on room air, forearm arterial vascular resistance was recorded during 30 min before phentolamine infusion and then an additional 30 min after its initiation before hypoxic exposure. During each of these 30-min periods, forearm vascular resistance (Fig. 1) remained stable. We are, therefore, confident that no added effects of continuous phentolamine infusion occurred on forearm vascular flow after the loading dose. Arterial blood pressure and heart rate did not change, and the control arm blood flow remained stable after phentolamine infusion, providing reassurance that we did not produce systemic effects with the drug infusion.

Finally, two of our subjects were overweight, and thus they were at risk for sleep-disordered breathing. Our protocol did not include a full-night polysomnography, and we cannot rule out occult obstructive sleep apnea. Blunted vasodilation has been demonstrated in obstructive sleep apnea patients (11, 17). Despite their high body mass index (28.72 and 29.86 kg/m\(^2\)), our two overweight subjects denied symptoms of sleep-disordered breathing and demonstrated similar forearm vascular resistance responses to our protocol as those subjects with body mass indexes of \(< 25 \text{ kg/m}^2\).

Whatever the mechanism by which vascular resistance was decreased after hypoxia, this vasodilation was totally counteracted by sympathetic tone, as demonstrated by the complete return to baseline of blood flow in the control forearm. We hypothesize, then, that persistent vasodilation is likely to account for sustained sympathoexcitation after hypoxic exposure. The metaboreflex, and the cardiopulmonary and arterial baroreflexes, may all be engaged to increase sympathetic tone in response to systemic vasodilator release (4, 6, 12). The metaboreflex is a major component in the increase of sympathetic vasomotor outflow in exercising humans (4). In our study, we did not investigate this sympathetic pathway and any metabolite that might accumulate in tissues after hypoxia. Additionally, because we did not quantify central venous pressure, we cannot discount cardiopulmonary baroreflex engagement, because our study concentrated on arterial vascular flow. Hansen et al. (9) did measure how much the cardiopulmonary reflex may be engaged in the MSNA increases occurring in healthy subject after 4 wk of hypoxic altitude exposure. The slight reduction in MSNA that they found after serum saline bolus infusion argues against major control of the MSNA by the cardiopulmonary reflex after long-term hypoxic exposure. Recent work from Halliwill et al. (8) demonstrates that, during short-term isocapnic hypoxia, there is a resetting of the baroreflex to higher blood pressure, heart rate, and sympathetic outflow without a change in baroreflex sensitivity. This resetting occurs during isocapnic hypoxia, but not during isocapnic hyperpnea, meaning that it does not arise from increased ventilation but is instead a result of direct peripheral chemoreceptor activation by hypoxia (8). This resetting also results in a higher MSNA and heart rate for a given arterial blood pressure.

Interestingly, our results demonstrated a persistent increase in heart rate without change in arterial blood pressure during hypoxia and subsequent recovery. Although we believe that the persistent increase in sympathetic outflow after hypoxia occurs for some part via the baroreflex, we have to acknowledge that our work does not provide a demonstration of baroreflex engagement but only a hypothesis mediated by the persistent forearm arterial vasodilation after hypoxia. Arguing against the arterial baroreflex engagement hypothesis, however, is the absence of change in arterial pressure during recovery from hypoxia. Two possible physical mechanisms might be advanced to explain baroreflex engagement after hypoxia in the absence of a measurable change in arterial pressure. It should be acknowledged that the baroreflex is engaged by a decrease in stretch of the baroreceptor, rather than by a decrease in arterial pressure. Although arterial pressure did not change in our subjects, we did observe an increase in heart rate. The increase in heart rate, combined with a decrease in vascular resistance, suggests that cardiac output may have increased, which may increase blood flow velocity. This increase in blood flow velocity may produce carotid diameter change by a simple...
physical mechanism. Total pressure in one point of a vessel is the sum of static pressure and dynamic pressure, if the pressure related to fluid viscosity remains negligible. When fluid velocity increases, dynamic pressure rises with a resultant drop in static pressure. Static pressure affects vessel diameter, so this drop may decrease the carotid diameter and engage the baroreflex to stimulate increased sympathetic outflow. This hypothesis could be investigated by direct visualization of carotid diameter during and after hypoxic exposure, a study not yet done to our knowledge. Indirect support for this hypothesis is provided, however, by the changes in sympathetic activity that have been observed after gradually increasing doses of intravenous sodium nitroprusside. Low-dose nitroprusside, at levels insufficient to produce a change in arterial pressure, nevertheless activates peripheral sympathetic activity (10). Although these mechanisms have been previously described (10), their role in baroreflex engagement in the recovery period from short-term hypoxia requires further study to be demonstrated.

In conclusion, our findings support the hypothesis that an active arterial vasodilator mechanism occurs in the forearm muscle during, and is still effective 30 min after, a brief isocapnic hypoxic exposure. This prolonged vasodilation may explain the sustained sympathoexcitation observed by others after a brief exposure to hypoxia.

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