Hyperventilation and human calf blood flow

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COFFMAN, JAY D., AND PETER KELLY. Hyperventilation and human calf blood flow. Am. J. Physiol. 211(5): 1255-1260. 1966.—Calf blood flow was measured in normal subjects by venous occlusion plethysmography, and capillary blood flow by the disappearance rate of an intramuscular injection of NaI131 before and during hyperventilation at 30 breaths/min for 3 min. Hyperventilation with room air produced a significant increase in plethysmographic calf blood flow, a decrease in vascular resistance, and an increase in the disappearance rate of NaI131. Hyperventilation with 5% carbon dioxide in oxygen produced no significant change in calf blood flow, vascular resistance, or NaI131 disappearance rate. After 10 mg propranolol was injected intravenously, a significant decrease occurred in calf blood flow but not in vascular resistance; the vasodilatation previously induced by intravenous epinephrine was replaced by vasoconstriction. Propranolol failed to completely block the increase in calf blood flow and decrease in vascular resistance during hyperventilation, although a significant but small attenuation did occur. It is concluded that hyperventilation produces an increase in muscle capillary blood flow which is partially but not entirely mediated by stimulation of beta receptors.

Hyperventilation causes total blood flow to increase and vascular resistance to decrease in the forearm and calf of human subjects (1-3,8). The changes are considered to be in the muscular bed since hand blood flow (representing skin) decreases during hyperventilation (1, 3, 8). Whether the increase in blood flow represents an increase in capillary flow to muscle or flow shunted through arteriovenous channels has not been studied. The mechanism producing the increase in blood flow also remains unknown. Clarke (3) and Roddie, Shepherd, and Whelan (8) have commented that the increase in blood flow is most likely secondary to a humoral mechanism. Clarke also compared the striking resemblance of the blood flow response during hyperventilation to that produced by intra-arterial infusion of epinephrine. The concomitant decrease in skin circulation would also be consistent with the action of epinephrine (6).

In the present investigation, the disappearance rate of a radioisotopic injected into skeletal muscle was studied during hyperventilation to determine if the increase in blood flow was in skeletal muscle and if the blood flow was through capillary or through arteriovenous anastomotic channels. The effect of beta receptor blockade by propranolol on the blood flow response was also studied to determine if beta receptor stimulation was involved.

METHODS

Total calf blood flow was measured by venous occlusion plethysmography on lightly clothed subjects lying in the supine position. The subject’s calf was slightly elevated and enclosed in a plethysmograph filled with water at a temperature of 34°C. This position maintained the posterior aspect of the lower leg approximately at heart level. The technic for enclosing the limb in the water plethysmograph has been described previously in detail (12). The water level within the plethysmograph (10 cm above the calf) produced a hydrostatic pressure equal to or slightly greater than normal local venous pressure. The foot circulation was excluded during flow measurements by inflating an 8-cm wide pneumatic cuff immediately distal to the plethysmograph to 50 mm Hg above the subject’s systolic pressure, as measured in the arm by the auscultatory method. A second pneumatic cuff, 13 cm wide, was placed just above the knee proximal to the plethysmograph. Inflation of this cuff produced the venous occlusion necessary to measure blood flow. The lowest venous occlusion required to obtain the maximum rate of increase in calf volume was determined at the beginning of each experiment and averaged 30 mm Hg. Resulting fluctuations in water level were detected by a Sanborn displacement transducer which senses the vertical motion of a 4-inch diameter Lucite float. The transducer was used in conjunction with a Sanborn strain-gauge amplifier and a direct-writing recorder. The recording system was calibrated at the beginning and end of each experiment by introducing known quantities of water...
into the plethysmograph. The volume of the calf within the plethysmograph was determined after the experiment by measurement of the water displaced.

The disappearance rate of a radioisotope from the lateral calf muscle of the other leg was used as a measure of skeletal muscle capillary blood flow (4). An injection of 0.1 ml NaI$^{131}$ in saline was made with a 26 gauge 5/8-inch needle approximately 3 inches below the head of the fibula; the needle was inserted to its hub in an attempt to control depth of injection. The disappearance rate was monitored by a shielded scintillation probe, ratemeter (R.E.A.C. model H580; time constant set at 10 sec), and linear recorder. The scintillation probe contained a 1 X 1 inch thick sodium iodide crystal, thallium activated. The dose of NaI$^{131}$ varied from 5 to 10 μC. Disappearance rates were plotted on semilogarithmic paper after subtraction of the background counts. Disappearance rates are expressed as half-times. An increase in the half-time was accepted as indicating a fall in capillary blood flow; conversely, a decrease in the half-time indicated a rise in capillary blood flow.

Blood flows and disappearance rates were recorded before, during, and after hyperventilation. Three control blood flows were obtained every 5 min until they became stable, and radioactive counts were followed for 5 to 10 min to assure a linear disappearance rate. Subjects then hyperventilated at 30 moderately deep breaths per min (timed by a stopwatch). A pneumotachograph around the subject's chest was used to record respiratory rate. In experiments in which subjects hyperventilated a gas mixture of 5% carbon dioxide in oxygen, a mouthpiece, nose clamp, and two-way valve were used; this apparatus was also used for room air hyperventilation in these experiments.

Ten subjects hyperventilated before and 45 min after an intravenous injection of 10 mg propranolol. Propranolol and epinephrine hydrochloride were injected via the tubing of a slow intravenous infusion of saline which ran throughout the experiment. Epinephrine was given by a constant-infusion pump at a rate of 10 μg/ml per min for a 5-min period both before and 25 min after the propranolol injection.

Sphygmomanometric blood pressure determinations were followed every 5 min during the control periods, every 30 sec during hyperventilation, and every 2 min during epinephrine administration. Mean blood pressure was calculated by adding one-third of the pulse pressure to the diastolic pressure. Vascular resistance was obtained by dividing the mean blood pressure by the blood flow and was expressed in arbitrary units.

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Total blood flow was expressed in milliliters per 100 ml of tissue per minute; disappearance rates were expressed as half-times. In the tables, mean values and their standard errors are given. Statistical analyses were performed using the Student t test method with each individual serving as his own control (10). A probability level of less than 0.05 was considered significant.

**RESULTS**

**TABLE 1. Hyperventilation (HV) with room air and 5 % CO₂**

<table>
<thead>
<tr>
<th></th>
<th>Calf Vascular Resistance, units</th>
<th>Calf Blood Flow, ml/100 ml per min</th>
<th>Blood Pressure, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control before HV</td>
<td>26.3±2.2</td>
<td>3.6±0.3</td>
<td>86.9±1.3</td>
</tr>
<tr>
<td>Room air HV</td>
<td>19.6±1.0*</td>
<td>5.0±0.5*</td>
<td>89.2±1.6†</td>
</tr>
<tr>
<td>Control before HV</td>
<td>50.1±2.6</td>
<td>3.0±0.2</td>
<td>85.9±2.8</td>
</tr>
<tr>
<td>Carbon dioxide HV</td>
<td>29.5±1.0†</td>
<td>3.5±0.3†</td>
<td>95.7±2.0*</td>
</tr>
</tbody>
</table>

All values given are ±SE. Control calf vascular resistances and blood flows determined immediately before the start of hyperventilation. *P < 0.001. †P < 0.02. ‡P < 0.5.
HYPERVENTILATION AND CALF BLOOD FLOW

jects (P < 0.001). Following hyperventilation, NaI\textsuperscript{131} disappearance rates slowed to an average of 16.6 ± 1.8 min which was also significantly different from the hyperventilation half-times (P < 0.001). Systemic blood pressure did not change significantly during hyperventilation.

Figure 1 depicts the plethysmographic calf blood flow and NaI\textsuperscript{131} disappearance rate in one experiment. Total calf blood flow showed an initial large increase during hyperventilation followed by a smaller rise. The NaI\textsuperscript{131} disappearance rate half-time of 13.1 min increased to 16.5 min during hyperventilation, the half-time slowed to 16.5 min.

**Hyperventilation with 5% CO\textsubscript{2}**. Hyperventilation with a gas mixture of 5% CO\textsubscript{2} in 95% oxygen did not produce a significant increase in calf blood flow or decrease in vascular resistance (Table 1). Five of the ten subjects showed no increase in calf blood flow, and four showed no decrease in resistance. Calf flow averaged 3.0 before and 3.5 ml/100 ml per min during hyperventilation for all 10 subjects tested. Calculated vascular resistance averaged 30.1 units before and 29.5 units during hyperventilation. The NaI\textsuperscript{131} disappearance rate from calf muscle was studied in four subjects during 5% CO\textsubscript{2} hyperventilation; no changes in the disappearance rates were seen. A significant rise in systemic blood pressure occurred during hyperventilation with CO\textsubscript{2}. Figure 2 depicts the plethysmographic calf blood flow and NaI\textsuperscript{131} disappearance rate during hyperventilation with room air and then with CO\textsubscript{2} in one experiment. The NaI\textsuperscript{131} disappearance rate and calf blood flow increase during hyperventilation with room air; no increase is seen during hyperventilation with CO\textsubscript{2}.

**Table 2. Hyperventilation (HV) with room air before and after propranolol**

<table>
<thead>
<tr>
<th></th>
<th>Calf Vascular Resistance, units</th>
<th>Calf Blood Flow, ml/100 ml per min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Before propranolol</td>
<td>23.8 ±3.2</td>
</tr>
<tr>
<td></td>
<td>After propranolol</td>
<td>26.3 ±2.3</td>
</tr>
<tr>
<td>Control</td>
<td>Before HV</td>
<td>20.8 ±3.9</td>
</tr>
<tr>
<td></td>
<td>HV</td>
<td>17.7 ±1.5</td>
</tr>
<tr>
<td>After propranolol</td>
<td>Control before HV</td>
<td>20.8 ±2.3</td>
</tr>
<tr>
<td></td>
<td>HV</td>
<td>22.5 ±1.1</td>
</tr>
</tbody>
</table>

All values given are ±SE. Control calf vascular resistances and blood flows determined immediately before the start of hyperventilation. *P < 0.1, †P < 0.05, ‡P < 0.001.
Hyperventilation with room air after propranolol: Ten subjects were given propranolol intravenously. A significant decrease occurred in calf blood flow which averaged 4.2 before and 3.5 ml/100 ml per min after propranolol, but the increase in vascular resistance after propranolol to 26.8 from 23.8 units was not statistically significant (Table 2). In a previous study, calf blood flow was found to decrease significantly as experiments progressed when only placebo were administered (5).

Significant decreases in calf vascular resistance and increases in calf blood flow occurred during hyperventilation both before and after the administration of propranolol (Table 2). Therefore, the beta receptor blocker as used did not prevent the vasodilatation caused by hyperventilation. However, 9 of the 10 subjects studied with calf flows showed a larger calf vascular resistance and a smaller blood flow during hyperventilation after, than before propranolol. The calf vascular resistance during hyperventilation of 17.7 units before propranolol was significantly different from the resistance of 22.5 units during hyperventilation after propranolol. Calf blood flow during hyperventilation before propranolol also differed significantly from the flow during hyperventilation after propranolol administration. Since calf blood flow had decreased during the course of the experiments, percentage change in blood flow during hyperventilation (hyperventilation blood flow divided by control blood flow × 100) was also calculated and compared before (47.8% increase) and after (26.0% increase) propranolol; a significant difference was found (P < 0.05). Therefore the beta receptor blocker as used did attenuate the effect of hyperventilation on calf blood flow and vascular resistance.

The 10 subjects also received intravenous epinephrine before and after propranolol to document blockade of beta receptors. Calf blood flow increased in all subjects during the 5-min epinephrine infusion before propranolol but decreased in all subjects after propranolol. Calf vascular resistance decreased an average of 7.7 units before as compared with an increase of 34.8 units after propranolol (P < 0.001). Therefore, beta receptor blockade appeared to be present in these experiments.

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Figures 3 and 4 depict two experiments in which the subjects hyperventilated room air and received intravenous epinephrine before and after propranolol. In both figures, it can be seen that, following propranolol administration, epinephrine produced vasoconstriction instead of vasodilatation. After propranolol, a large rise in systemic mean blood pressure occurred during epinephrine infusions. In Fig. 3 it can be seen that propranolol produced little change in the increase in calf blood flow which occurred during hyperventilation; in Fig. 4, a definite attenuation is seen.

Systemic blood pressure changes during hyperventilation averaged an increase of 5 mm Hg before propranolol and 3.9 mm Hg after propranolol; there was no significant difference (P < 0.4). Subjects experienced no symptoms from the propranolol except for burning in
HYPERVENTILATION AND Calf Blood Flow

HYPERVENTILATION

Room Air

EPINEPHRINE I.V.

EPINEPHRINE

HYPERVENTILATION

Room Air

PROPRANOLOL 0.1 mg I.V.

MEAN B.P. mmHg

50 sec

10 sec

30 sec

50 sec

10 sec

30 sec

50 sec

10 sec

30 sec

50 sec

10 sec

30 sec

FIG. 4. Plethysmographic calf blood flow in one experiment in which the subject hyperventilated room air and received iv epinephrine before and after propranolol iv. A definite attenuation in the increase in calf blood flow during hyperventilation is seen after propranolol. Following propranolol, calf blood flow decreased during epinephrine infusion whereas a large increase in flow occurred before propranolol.

Discussion

In the present and previous studies (1-3, 8), hyperventilation produced a significant increase in plethysmographic calf and forearm blood flow. The increase in the disappearance of NaI131 from the calf muscle during hyperventilation demonstrates that the increase in blood flow occurs in muscle. Previously, it was surmised that the increase in calf or forearm blood flow was in the muscular bed since skin (hand) blood flow (1, 3, 8) was shown to decrease during hyperventilation. The increase in the disappearance rate of the radioisotope during hyperventilation also indicates that an increase in capillary blood flow is involved.

The mechanism of vasodilatation in muscle during hyperventilation has not been completely elucidated. The mechanical, nervous, and chemical alterations that occur during hyperventilation have been investigated. In the present study, a significant change did not occur in systemic blood pressure during hyperventilation.

Other investigators have reported a fall (2, 3, 8), no change (7), or a rise in blood pressure (1) during hyperventilation. There has also been a difference in the reported findings regarding cardiac output (2, 9). Therefore, it would be difficult to explain the increase in muscle blood flow by a change in blood pressure or cardiac output in all studies.

In the present and previous studies (3, 8), hyperventilation using 5% carbon dioxide induced no change or smaller increases in muscle blood flow than hyperventilation with room air. Therefore the increase in blood flow cannot be entirely a consequence of intrathoracic pressure changes and must be related in part to the resulting hypocapnia. The hypocapnia could produce its effect via the nervous system, by a direct vascular effect, or by release of a humoral substance. During hyperventilation with room air, alveolar carbon dioxide is lowered (3), arterial pH rises, and carbon dioxide tension decreases (7). Since an increase in forearm and a decrease in hand blood flow occur during hyperventilation even after nerve block or sympathectomy of the arm (2, 8), the muscle vasodilatation and cutaneous vasoconstriction presumably are not effected via the nervous system. Locally, low carbon dioxide tension with an increased pH produces muscle vasodilatation (7); the local effect of low carbon dioxide tension with no change in pH is not known. Since vasoconstriction occurs in the skin during hyperventilation, any chemical change in the blood responsible for the vascular changes would have to affect skin and muscle blood vessels differently.

The release of epinephrine from the adrenal medulla by hypocapnia would cause cutaneous vasoconstriction and muscle vasodilatation. Clarke (3) first commented on the remarkable similarity of the forearm blood flows during hyperventilation to the blood flow increases produced by intravenous epinephrine (a large initial vasodilatation followed by a lesser increase in blood flow). Although the vasodilator effect of epinephrine was effectively blocked in the present experiments, propranolol failed to block completely the increase in muscle blood flow during hyperventilation. Significant, sizeable increases in blood flow and decreases in vascular resistance still occurred. However, propranolol did cause small but significant attenuation of the effect of hyperventilation on muscle blood flow and vascular resistance and therefore, it could be argued that epinephrine or another beta receptor stimulator might be involved in the response.

The mechanism of the increase in muscle blood flow with hyperventilation may, with further study, prove to be a combination of the complex factors occurring during hyperventilation as suggested by Roddie, Shepherd, and Whelan (8). As reported in the cited references, there is disagreement concerning the effects of hyperventilation on cardiac output and blood pressure. It has also been shown that hyperventilation with carbon dioxide does not always completely block the increase...
in blood flow. The effect of hyperventilation on muscle blood flow may result from a combination of mechanisms including low arterial CO₂ tension and increased pH, circulating vasodilator substances, and changes in cardiac output, blood pressure, or intrathoracic pressure. Each of these factors may assume a different degree of importance depending on how the hyperventilation has been performed; most studies have used different rates and depths of respiration during hyperventilation and therefore may have induced increases in muscle blood flow by different mixtures of these mechanisms.

This study was performed with the valuable technical assistance of Mrs. Beverly Hull, R.N. The propranolol (AY-64043) used in this study was kindly supplied by Alex Sahagian-Edwards, M.D., Assistant Medical Director, Medical Department, Ayerst Laboratories, New York City.

REFERENCES