CONTRIBUTION OF HYPERCAPNIA AND HYPOXIA TO THE VASCULAR RESPONSE TO ISCHAEMIA

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SUMMARY

1. Hypoxia, induced by 7–12% oxygen breathing, produced vasodilatation in the intact or in the phenoxybenzamine and propranolol treated forearm of human volunteers when arterial blood $PO_2$ decreased below 45 mmHg, or when deep forearm venous blood $PO_2$ decreased below 35–40 mmHg.

2. Circulatory arrest of the forearm following alpha and beta adrenergic receptor blockade was followed by greater increases in blood flow and greater decreases in forearm vascular resistance during $CO_2$ breathing than during room air breathing. The increased flow following ischaemia was maintained at a high level until $CO_2$ administration was stopped.

3. The vasodilator response following ischaemia of the human forearm, produced by digital occlusion of the brachial artery, was compared to that produced by hypercapnia or hypoxia or a combination of the two, produced by breathing the appropriate gas mixtures. The forearm was pre-treated with phenoxybenzamine and propranolol to produce alpha and beta adrenergic receptor blockade. For equal increases in deep forearm venous blood $PCO_2$, the vasodilator response to hypercapnia averaged 60% of that following ischaemia. For equal decreases in deep forearm venous blood $PO_2$ the vasodilator response to hypoxia averaged 26% of that produced by ischaemia. The vasodilator response to ischaemia was not modified by breathing 100% oxygen to maintain the deep forearm venous blood $PO_2$ at a level above that seen with the circulation free during room air breathing. Combined hypoxia and hypercapnia of equal severity as those produced by ischaemia resulted in a vasodilator response which averaged 64% of that produced by ischaemia.

The purpose of the present investigation was threefold: to examine the effect of hypoxia on human forearm blood vessels, to study the influence of increased blood and tissue carbon dioxide tension on reactive hyperaemia of the human forearm and to evaluate the contribution...
of hypercapnia and hypoxia to the vasodilator response to ischaemia of the human forearm.

The effect of hypoxia on limb blood vessels has been examined in animals and in man (Abramson, Landt, & Benjamin, 1943; Anderson, Allen, Barcroft, Edholm & Manning, 1946; Black & Roddie, 1958; Chalmers, Korner & White, 1966; Daugherty, Scott, Dabney & Haddy, 1967; Graf, 1965; Richardson, Kontos, Raper & Patterson, 1967; Ross, Fairchild, Weldy & Guyton, 1962). There is general agreement that hypoxia, if sufficiently severe, is vasodilator. The quantitative aspects of hypoxic vasodilatation were examined by only a few investigators in the dog. Ross et al. (1962) found pronounced vasodilatation in the perfused hindlimb of anaesthetized dogs in response to mild or moderate hypoxia. They suggested that hypoxia was a major factor in the local regulation of blood flow. Daugherty et al. (1967) found no change in vascular resistance of the perfused forelimb of anaesthetized dogs until arterial blood \( PO_2 \) was reduced below 30 mmHg. They concluded that hypoxia could not be the sole cause of all autoregulatory phenomena, and that its role in the local regulation of blood flow could only be considered in those physiological conditions where oxygen tension in the environment of the high resistance vessels decreased below 30 mmHg. The reasons for these divergent results are not entirely clear. It appeared important, however, to re-examine the quantitative aspects of the vasodilator response to hypoxia in a vascular bed with high vascular tone, preferably without possible modifying effects from anaesthesia and surgery.

Fairchild, Ross & Guyton (1966) observed that perfusion of the limb in anaesthetized dogs with blood having zero oxygen saturation following a 10-min period of ischaemia resulted in a sustained increase in flow, without overshoot, which was maintained until the limb was again perfused with blood containing oxygen. They interpreted these findings as indicating that oxygen was necessary for recovery from reactive hyperaemia. They also suggested that the absence of an overshoot in blood flow under these circumstances indicated that vasodilator metabolites were not involved in the production of the hyperaemic response to ischaemia. Since previous work from our laboratory (Kontos & Patterson, 1964; Kontos, Mauck & Patterson, 1965) suggested that carbon dioxide might be important in the production of the vasodilator response to ischaemia we tested the possibility that increase in blood and tissue carbon dioxide tension might have the same effect on reactive hyperaemia as the absence of oxygen.

Local hypercapnia and local hypoxia have been traditionally suspected as playing a role in the production of the vasodilator response to ischaemia. Their possible contribution to this response has recently been re-emphasized (Kontos & Patterson, 1964; Kontos et al. 1965; Guyton, Ross, Carrier & Walker, 1964). Despite this, there are no studies in which the effects of hypoxia and hypercapnia have been compared to those produced by ischaemia. In the present investigation, the vasodilator effect of ischaemia of the human forearm was compared to that produced by hypercapnia or hypoxia or by a combination of the two, induced by breathing the appropriate gas mixtures.

METHODS

Experiments were done on eighty-eight normal, young volunteers. All studies were done in an air-conditioned laboratory (room temperature 23–24°) with the subjects recumbent on a table. Studies on the same subject were made at least 2 weeks apart.

Forearm blood flow was measured by venous occlusion plethysmography, using water-
Hypercapnia and hypoxia on limb blood flow

filled plethysmographs whose temperature was maintained thermostatically at 34°. The circulation through the hand was excluded by inflating a wrist-cuff to a pressure well above the subject's systolic blood pressure. Arterial blood pressure was measured with a Statham P-23 Db strain-gauge connected to a Teflon catheter placed in the brachial artery at the elbow. Forearm vascular resistance was calculated by dividing mean arterial blood pressure by forearm blood flow. Venous blood was obtained from another Teflon catheter passed upstream into the deep branch of the median cubital vein. Blood obtained from this vein is known to come primarily from skeletal muscle (Coles, Cooper, Mottram & Oclewhaw, 1958). The gas tensions of arterial and venous blood were measured with electrodes at 37° (Severinghaus & Bradley, 1958). Venous blood gas tensions were corrected to the temperature of the blood at the time of collection as described elsewhere (Kontos & Patterson, 1964). Expired air CO₂ concentration was monitored throughout the experiment with a Beckman infra-red CO₂ analyzer. In several experiments the vasoconstrictor effect of increased activity of sympathetic nerves and of circulating catecholamines and the vasodilator effect of catecholamines were abolished by the intra-arterial administration of phenoxybenzamine and propranolol, as described previously (Kontos, Richardson & Patterson, 1967). Phenoxybenzamine (8–12 mg) and propranolol (0.3–0.5 mg) were separately dissolved in 10 ml of 0-9% NaCl solution and given into the brachial artery over a period of 5 min. The effectiveness of α-adrenergic blockade was verified by showing that the increase in vascular resistance which normally accompanies the post-Valsalva manoeuvre overshoot in blood pressure was abolished. It is known that this increase in vascular resistance is mediated by increased activity of sympathetic vasoconstrictor nerve fibres (Roddie, Shepherd & Whelan, 1958). The effectiveness of β-adrenergic blockade was shown at the end of the experiment by the absence of the usual vasodilator effect of intravenous epinephrine (10 μg/min).

In fifty-two subjects the effect of hypoxia on forearm blood flow was studied. Hypoxia was induced by breathing 7–12% oxygen-in-nitrogen administered from a Douglas bag through wide-bore tubing and a low resistance valve connected to a mouthpiece. Hypoxia was maintained until stable levels of blood flow were obtained for at least 2 min. The duration of hypoxia ranged from 7 to 15 min.

The effect of CO₂ breathing on reactive hyperaemia was studied in seven subjects in the following manner. The circulation to the forearm was arrested by inflating a cuff placed at the upper part of the arm and inflated to 300 mmHg for 30 s. The first period of occlusion was carried out in the intact forearm and the results were discarded. Still using the intact forearm a second period of circulatory arrest for 30 s was carried out while the subjects breathed room air and again repeated after the forearm was treated with intra-arterial phenoxybenzamine and propranolol to produce α- and β-adrenergic receptor blockade. Another period of ischaemia was carried out 2 min after the onset of 9% CO₂ inhalation. The CO₂ inhalation was continued during the period of ischaemia and for several minutes after the occlusion was released. Measurements of forearm blood flow were made immediately following release of the occlusion of the forearm and at 15 s intervals thereafter. Rest periods of 10 min were allowed between arterial occlusions.

The contribution of hypoxia and hypercapnia to the vasodilator response to ischaemia were evaluated as follows. Alpha and beta-adrenergic receptor blockade were induced in the forearm and when forearm blood flow reached a stable level two to three sets of arterial and venous blood samples were obtained at 3 min intervals with intervening blood flow measurements.
Then the brachial artery was occluded by compressing the vessel by digital pressure against the humerus at the level close to the axilla. Care was exercised not to occlude the brachial vein and congest the forearm. If this occurred, it could readily be recognized from the increase in the plethysmographically measured forearm volume. The procedure was then repeated. Occlusion of the brachial artery was maintained for 5–7 min. Longer periods of occlusion were not possible because of clotting of the intra-arterial catheter and inability of the experimenter to maintain uniform compression of the artery. Patency of the arterial catheter was considered essential in order to be certain that uniform compression of the vessel was maintained, as judged by a stable level of arterial blood pressure distal to the occlusion. During the period of occlusion, 10 to 15 venous blood samples were obtained for PO₂ and PCO₂ determinations. Blood flow measurements were resumed immediately following release of the brachial arterial occlusion. Upon release of the arterial occlusion, forearm blood flow increased above its level seen in the control period (reactive hyperaemia) and then declined rapidly to the control level (Fig. 1). The maximum decrease in forearm vascular resistance in the early post-occlusion period was selected for comparison instead of the change in resistance during ischaemia for the following reasons: blood flow measurements during the period of ischaemia might be of uncertain reliability because of the low arterial pressure; frequent venous occlusions might alter the venous blood gas tensions; over the range of arterial pressures observed, the pressure-flow relationship of the vascular bed of skeletal muscle is curvilinear (Green, Rapela & Conrad, 1963). Therefore, comparison of calculated vascular resistance during ischaemia to values calculated when the circulation was free might be difficult to interpret. It is recognized, however, that this choice would result in overestimation of the decrease in resistance attributable to decreased vascular tone as a result of ischaemia. This is due to the fact that when the arterial occlusion was released arterial blood pressure increased suddenly, and it is likely that this would distend the vessels passively. It must also be noted that, because blood flow is declining rapidly during the immediate post-ischaemic period, it is easy to miss the peak blood flow. This would result in underestimation of the vasodilator response to ischaemia and hence in overestimation of the contributions of hypercapnia and hypoxia to this response.

It was assumed that the changes in venous blood gas tensions would represent satisfactory approximations of the changes in tissue gas tensions, if a steady state with respect to oxygen and CO₂ could be achieved during ischaemia. For this reason the experiments included in the results fulfilled the following criteria: the PO₂ and PCO₂ of all blood samples obtained during the last 2 min of ischaemia did not differ from the corresponding mean tension during this period by more than 2 mmHg. It should be noted, however, that fulfilment of these criteria, although providing satisfactory guard against large deviations from the steady state, does not assure with complete certainty establishment of a steady state, especially with regard to CO₂ for which the mean transit time through limb tissues is probably very long (Coxon & Robinson, 1959).

After a 10-min rest period, control measurements of blood flow and blood gas tensions were repeated and then one of four procedures was carried out. (1) Subjects breathed CO₂ gas mixtures in a concentration sufficient to produce the same increase in venous blood CO₂ tension as that produced by ischaemia. The selection of the appropriate CO₂ gas mixture was aided by experience obtained in previous experiments in which the effect of CO₂ breathing on forearm blood flow was studied and the changes in deep forearm venous blood CO₂ tension measured (Kontos et al., 1967). In practice, two or three different concentrations of CO₂,
Hypercapnia and hypoxia on limb blood flow

Differing by 0.5–1%, were given sequentially. Each concentration was breathed about 20 min. Blood flow measurements and blood samples were obtained during the last 5 min of breathing a given gas mixture. The changes in vascular resistance produced by the gas mixture which gave an increment in venous blood PCO₂ closest to that found during ischaemia was selected for comparison. (2) Subjects breathed gas mixtures containing 9–12% oxygen to produce the same decrease in deep venous forearm blood oxygen tension as that found during ischaemia. (3) Subjects breathed gas mixtures containing low concentrations of O₂ to which sufficient CO₂ was added to reproduce both the decrease in venous blood PO₂ and the increase in venous blood PCO₂ seen during ischaemia. (4) Subjects were given 100% oxygen to breathe for 7 min and the occlusion of the brachial artery was repeated as before. The purpose of this was to study the effect of maintaining, during ischaemia, venous blood oxygen tension above its value seen with the circulation free when the subjects breathed room air.
RESULTS

Effect of hypoxia on forearm blood flow

Only experiments in which hypoxia was well tolerated by the subjects and in which stable levels of flow were achieved were considered in the results. Experiments in which blood flow was variable throughout hypoxia or in which hypotensive episodes occurred were excluded from further consideration.

In thirty-eight experiments blood flow in the intact forearm reached stable levels during hypoxia. The results of these experiments are summarized in Table 1. There was a significant increase in forearm blood flow and a significant decrease in forearm vascular resistance.

<table>
<thead>
<tr>
<th></th>
<th>Room air</th>
<th>Hypoxia</th>
<th>Difference</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBF (ml min⁻¹ 100 ml⁻¹)</td>
<td>2.6±0.2</td>
<td>3.4±0.2</td>
<td>0.8±0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MABP (mmHg)</td>
<td>92.9±1.6</td>
<td>91.9±1.7</td>
<td>1.0±0.8</td>
<td>NS</td>
</tr>
<tr>
<td>FVR (mmHg ml⁻¹ min 100 ml)</td>
<td>41.5±3.0</td>
<td>32.6±2.7</td>
<td>8.9±1.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PaO₂ (mmHg)</td>
<td>93.9±1.1</td>
<td>38.2±1.7</td>
<td>55.7±1.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PaCO₂ (mmHg)</td>
<td>38.6±0.5</td>
<td>31.3±0.6</td>
<td>7.3±0.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PvO₂ (mmHg)</td>
<td>41.2±2.4</td>
<td>27.5±2.2</td>
<td>13.7±2.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PvCO₂ (mmHg)</td>
<td>44.1±1.5</td>
<td>39.5±1.2</td>
<td>4.6±1.2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

All values are mean ± SEM. All values were obtained from thirty-eight experiments except PvO₂ and PvCO₂ which were obtained from twelve experiments. FBF: forearm blood flow; MABP: mean arterial blood pressure; FVR: forearm vascular resistance; PaO₂ and PaCO₂: arterial blood oxygen and CO₂ tensions; PvO₂ and PvCO₂: deep forearm venous blood oxygen and CO₂ tensions; NS: not significant. P refers to comparison of mean difference between room air and hypoxia values to zero by means of t-test.

Arterial blood pressure did not change significantly. As a result of the associated hyperventilation arterial blood PCO₂ decreased. The relationship between forearm vascular resistance during hypoxia, expressed as a percentage of the control value, and the associated arterial blood oxygen tension in these experiments is shown in Fig. 2. Vasodilator responses were observed when arterial blood oxygen tension decreased below 40-45 mmHg. There was considerable variation in the response shown by different subjects.
In twenty-eight experiments, blood flow in the forearm with α- and β-adrenergic receptor blockade reached stable levels during hypoxia. This was achieved between the fifth and tenth minute of hypoxia. The results of these experiments are summarized in Table 2. As in the intact forearm, there were significant increases in forearm blood flow and significant decreases in forearm vascular resistance. Arterial blood pressure did not change significantly and arterial blood $PCO_2$ decreased significantly. Forearm vascular resistance during hypoxia in these experiments with respect to the arterial and deep forearm venous blood $PO_2$ is shown in Figs. 3 and 4. As in the intact forearm, there was progressively greater decrease in vascular resistance with diminishing arterial or venous blood $PO_2$. Vasodilator responses were observed when arterial blood $PO_2$ decreased below 45 mmHg, or when deep forearm venous blood $PO_2$ decreased below 35–40 mmHg.

The response of blood flow to the forearm with α- and β-adrenergic receptor blockage to hypocapnic hypoxia was compared to that produced by hypoxia without hypocapnia, induced by breathing low oxygen to which sufficient carbon dioxide was added to maintain arterial
### Table 2. Effect of hypoxia on blood flow and vascular resistance of the forearm with α- and β-adrenergic blockade

<table>
<thead>
<tr>
<th></th>
<th>Room air</th>
<th>Hypoxia</th>
<th>Difference</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBF (ml min⁻¹ 100 ml⁻¹)</td>
<td>7.8 ± 1.0</td>
<td>9.0 ± 0.9</td>
<td>1.2 ± 0.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>MABP (mmHg)</td>
<td>93.3 ± 2.1</td>
<td>92.1 ± 1.7</td>
<td>1.2 ± 0.9</td>
<td>NS</td>
</tr>
<tr>
<td>FVR (mmHg ml⁻¹ min 100 ml)</td>
<td>16.0 ± 1.5</td>
<td>13.0 ± 1.1</td>
<td>3.0 ± 0.7</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>$P_{a, O_2}$ (mmHg)</td>
<td>96.1 ± 1.6</td>
<td>42.3 ± 2.1</td>
<td>53.8 ± 2.5</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>$P_{a, CO_2}$ (mmHg)</td>
<td>38.1 ± 0.5</td>
<td>31.9 ± 0.8</td>
<td>6.2 ± 0.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>$P_{v, O_2}$ (mmHg)</td>
<td>49.7 ± 2.3</td>
<td>33.2 ± 1.6</td>
<td>16.6 ± 2.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>$P_{v, CO_2}$ (mmHg)</td>
<td>40.8 ± 0.7</td>
<td>36.4 ± 0.8</td>
<td>4.4 ± 0.7</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

All values are mean ± SEM. All values were obtained from twenty-eight experiments except $P_{v, O_2}$ and $P_{v, CO_2}$ which were obtained from twenty-one experiments. Abbreviations and statistical analysis as in Table 1.

![Fig. 3](image-url)  
**Fig. 3.** Relationship between vascular resistance of the forearm during α- and β-adrenergic blockade with hypoxia, and the corresponding arterial blood oxygen tensions in twenty-eight experiments.
blood CO₂ tension close to the resting level. Because ventilation increases much more during hypoxia without hypocapnia than during hypocapnic hypoxia (Richardson, Kontos, Shapiro & Patterson 1966), in order to achieve comparable levels of arterial blood PO₂ during the two types of hypoxia, subjects breathed 9% oxygen during hypocapnic hypoxia and 7.5% oxygen when CO₂ was added to the inspired gas. The order of the two experiments was randomized. There was no significant difference in the response to the two types of hypoxia (Table 3).

![Fig. 4. Relationship between vascular resistance of the forearm during α- and β-adrenergic blockade with hypoxia and the corresponding deep forearm venous blood oxygen tensions in twenty-one experiments.](image)

**Effect of hypercapnia on reactive hyperaemia**

Following intra-arterial administration of propranolol and phenoxybenzamine, resting forearm blood flow increased by 150%, the maximum blood flow during reactive hyperaemia was higher by 67%, and the minimum forearm vascular resistance during reactive hyperaemia was reduced to 66% of the corresponding control value. The duration of reactive hyperaemia was not affected by α- and β-adrenergic receptor blockade (Table 4). During 9% CO₂ breathing forearm blood flow immediately following ischaemia increased by 55% and forearm vascular resistance was reduced by 30%. As illustrated in Fig. 5, in five of seven subjects forearm blood flow was maintained at approximately the same level as immediately following ischaemia for as long as CO₂ breathing was maintained (5–7 min). In the remaining two subjects forearm blood flow declined to approximately two-thirds of its value immediately following ischaemia and was maintained at that level for the remainder of the duration of CO₂ breathing.
### Table 3. Comparison of effect of hypoxia with and without hypocapnia on blood flow and vascular resistance of the forearm with $\alpha$- and $\beta$-adrenergic blockade

<table>
<thead>
<tr>
<th></th>
<th>Hypoxia without hypocapnia</th>
<th>Hypoxia with hypocapnia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>H</td>
</tr>
<tr>
<td>FBF (ml min$^{-1}$ 100 ml$^{-1}$)</td>
<td>7.3±1.3</td>
<td>9.5±1.3</td>
</tr>
<tr>
<td>MABP (mmHg)</td>
<td>89.4±2.2</td>
<td>90.8±3.1</td>
</tr>
<tr>
<td>FVR (mmHg ml$^{-1}$ min 100 ml)</td>
<td>14.7±2.2</td>
<td>10.9±1.6</td>
</tr>
<tr>
<td>$Pa_O_2$ (mmHg)</td>
<td>95.0±1.4</td>
<td>36.3±0.8</td>
</tr>
<tr>
<td>$Pa_CO_2$ (mmHg)</td>
<td>38.9±0.3</td>
<td>39.3±0.6</td>
</tr>
</tbody>
</table>

C: room air; H: hypoxia; D: difference between C and H. All values are mean±SEM obtained from eight experiments. Statistical comparison of D for hypoxia without and with hypocapnia by means of t-test showed no significant differences in any of the values. Abbreviations as in Table 1.

### Table 4. Comparison of reactive hyperaemia in the intact forearm during air breathing and in the forearm with $\alpha$- and $\beta$-adrenergic receptor blockade during air and during CO$_2$ breathing

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>RFBF (ml min$^{-1}$ 100 ml$^{-1}$)</td>
<td>3.5±0.7</td>
<td>8.8±1.3</td>
<td>9.8±1.4</td>
</tr>
<tr>
<td>RBP (mmHg)</td>
<td>97.7±3.9</td>
<td>102.1±3.1</td>
<td>115.1±5.9</td>
</tr>
<tr>
<td>RFVR (mmHg ml$^{-1}$ min 100 ml)</td>
<td>34.8±5.7</td>
<td>13.2±1.8</td>
<td>13.0±1.5</td>
</tr>
<tr>
<td>RHBF (ml min$^{-1}$ 100 ml$^{-1}$)</td>
<td>8.9±0.7</td>
<td>14.9±2.0</td>
<td>23.2±2.2</td>
</tr>
<tr>
<td>RHPB (mmHg)</td>
<td>97.1±3.6</td>
<td>101.7±3.0</td>
<td>115.1±5.8</td>
</tr>
<tr>
<td>RHVR (mmHg ml$^{-1}$ min 100 ml)</td>
<td>11.1±0.6</td>
<td>7.4±0.7</td>
<td>5.2±0.5</td>
</tr>
<tr>
<td>RHD (sec)</td>
<td>34.3±3.0</td>
<td>41.4±5.1</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: A: intact forearm, room air breathing; B: after $\alpha$- and $\beta$-adrenergic receptor blockade, room air breathing; C: after $\alpha$- and $\beta$-adrenergic receptor blockade, during CO$_2$ breathing; RFBF: resting forearm blood flow; RBP: resting mean arterial blood pressure; RFVR: resting forearm vascular resistance; RHBF: maximum forearm blood flow during reactive hyperaemia; RHPB: mean arterial blood pressure at time of maximum forearm blood flow during reactive hyperaemia; RHVR: minimum forearm vascular resistance during reactive hyperaemia; RHD: duration of increased flow during reactive hyperaemia. All values are mean±SEM from seven experiments on seven subjects.
Fig. 5. Response to 30-s period of circulatory arrest during room air breathing (A) and during 9% CO₂ breathing (B-D). MABP: mean arterial blood pressure; Pa,CO₂: end-tidal CO₂ tension. The numbers below each venous occlusion curve represent forearm blood flow in ml min⁻¹ 100 ml⁻¹. A shows the response to ischaemia during room air breathing. B and C are continuous and show the response to ischaemia during CO₂ breathing. D was obtained 5 min after the end of ischaemia just before CO₂ was stopped.
Contribution of hypercapnia and hypoxia to the vasodilator response to ischaemia

In five experiments blood was obtained from the arterial catheter before, during and after occlusion of the brachial artery by digital pressure. The PO₂ and PCO₂ of blood withdrawn during arterial occlusion were in all instances the same as before and after arterial occlusion, indicating that the blood supplied by collateral flow did not pass through vessels exchanging with metabolizing tissues.

The vascular response to ischaemia was compared to that produced by a comparable increase in venous blood PCO₂ induced by CO₂ breathing in eight experiments. Fig. 6 shows a typical experiment and Table 5 summarizes the results of these eight experiments. The increase in venous blood CO₂ tension during ischaemia and that seen during CO₂ breathing did not differ by more than 2 mmHg in any of the experiments. The decrease in vascular resistance produced by CO₂ breathing was less pronounced than that found immediately following ischaemia in all experiments and averaged 60% of that produced by ischaemia.

The response to breathing low oxygen-containing gas mixtures was successfully compared to that produced by ischaemia in seven experiments. Table 6 summarizes the results and Fig. 7 shows a typical experiment. The greatest difference between the decrease in venous blood PO₂ during ischaemia and that produced by hypoxia was 7.2 mmHg. The decrease in forearm vascular resistance produced by hypoxia averaged 26% of that seen immediately following ischaemia.
**Hypercapnia and hypoxia on limb blood flow**

**TABLE 5.** Comparison of vasodilator response to ischaemia to that produced by CO$_2$ breathing

<table>
<thead>
<tr>
<th></th>
<th>Ischaemia</th>
<th>CO$_2$ breathing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting MABP (mmHg)</td>
<td>90.6±3.7</td>
<td>87.0±2.5</td>
</tr>
<tr>
<td>Resting FBF (ml min$^{-1}$ 100 ml$^{-1}$)</td>
<td>6.4±0.9</td>
<td>5.5±0.4</td>
</tr>
<tr>
<td>Resting FVR (mmHg ml$^{-1}$ min 100 ml)</td>
<td>15.7±1.9</td>
<td>16.5±1.2</td>
</tr>
<tr>
<td>Resting $P_{\text{v},O_2}$ (mmHg)</td>
<td>44.8±1.5</td>
<td>43.6±1.6</td>
</tr>
<tr>
<td>Resting $P_{\text{v},CO_2}$ (mmHg)</td>
<td>40.5±0.7</td>
<td>41.0±0.9</td>
</tr>
<tr>
<td>MABP (during ischaemia or CO$_2$ breathing) (mmHg)</td>
<td>48.9±5.2</td>
<td>95.0±2.8</td>
</tr>
<tr>
<td>$P_{\text{v},O_2}$ (during ischaemia or CO$_2$ breathing) (mmHg)</td>
<td>32.2±0.4</td>
<td>51.8±3.1</td>
</tr>
<tr>
<td>$P_{\text{v},CO_2}$ (during ischaemic or CO$_2$ breathing) (mmHg)</td>
<td>45.7±0.9</td>
<td>46.0±0.9</td>
</tr>
<tr>
<td>FBF (maximum post-ischaemic or during CO$_2$ breathing) (ml min$^{-1}$ 100 ml$^{-1}$)</td>
<td>15.1±1.9</td>
<td>10.0±1.2</td>
</tr>
<tr>
<td>FVR (minimum post-ischaemic or during CO$_2$ breathing) (mmHg ml$^{-1}$ min 100 ml)</td>
<td>6.9±1.1</td>
<td>10.9±1.3</td>
</tr>
</tbody>
</table>

All values are mean ± SEM. Resting values were obtained during appropriate control periods with the circulation free and the subjects breathing room air.

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**Fig. 7.** Comparison of the response to 5 min brachial arterial occlusion (A), with response to hypoxia induced by prolonged breathing low concentrations of oxygen (B). Low oxygen breathing was started at the end of 7-min in panel B. Abbreviations as in Fig. 6.
TABLE 6. Comparison of vasodilator response to ischaemia to that produced by hypoxia

<table>
<thead>
<tr>
<th></th>
<th>Ischaemia</th>
<th>Hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting MABP (mmHg)</td>
<td>91.0 ± 6.1</td>
<td>91.0 ± 6.1</td>
</tr>
<tr>
<td>Resting FBF (ml min⁻¹ 100 ml⁻¹)</td>
<td>7.4 ± 1.1</td>
<td>7.4 ± 1.1</td>
</tr>
<tr>
<td>Resting FVR (mmHg ml⁻¹ min 100 ml)</td>
<td>13.3 ± 1.3</td>
<td>13.3 ± 1.3</td>
</tr>
<tr>
<td>Resting $P_v,O_2$ (mmHg)</td>
<td>47.5 ± 2.8</td>
<td>49.5 ± 3.2</td>
</tr>
<tr>
<td>Resting $P_v,CO_2$ (mmHg)</td>
<td>41.2 ± 1.4</td>
<td>40.9 ± 1.4</td>
</tr>
<tr>
<td>MABP (during ischaemia or hypoxia) (mmHg)</td>
<td>47.9 ± 4.8</td>
<td>88.9 ± 5.1</td>
</tr>
<tr>
<td>$P_v,O_2$ (during ischaemia or hypoxia) (mmHg)</td>
<td>29.4 ± 2.7</td>
<td>28.9 ± 1.3</td>
</tr>
<tr>
<td>$P_v,CO_2$ (during ischaemia or hypoxia) (mmHg)</td>
<td>46.4 ± 1.3</td>
<td>36.9 ± 1.7</td>
</tr>
<tr>
<td>FBF (maximum post-ischaemic or during hypoxia) (ml min⁻¹ 100 ml⁻¹)</td>
<td>16.0 ± 2.5</td>
<td>8.8 ± 1.6</td>
</tr>
<tr>
<td>FVR (minimum post-ischaemic or during hypoxia) (mmHg ml⁻¹ min 100 ml)</td>
<td>6.2 ± 0.8</td>
<td>11.5 ± 1.4</td>
</tr>
</tbody>
</table>

All values are mean ± SEM Abbreviations as in Table 1.

Fig. 8. Comparison of the response to ischaemia produced by brachial arterial occlusion during room air (A) and during 100% oxygen breathing (B). Note that the increase in forearm blood flow in response to ischaemia was the same despite the fact that venous blood oxygen tension was 18 mmHg higher in B than in A.
### Hypercapnia and hypoxia on limb blood flow

**Table 7. Comparison of vasodilator response to ischaemia during air and during 100% oxygen breathing**

<table>
<thead>
<tr>
<th></th>
<th>Room air</th>
<th>100%</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting MABP (mmHg)</td>
<td>89·2±2·7</td>
<td>90·0±3·2</td>
<td>NS</td>
</tr>
<tr>
<td>Resting FBF (ml mm⁻¹ 100 ml⁻¹)</td>
<td>6·9±1·1</td>
<td>6·0±0·9</td>
<td>&lt;0·02</td>
</tr>
<tr>
<td>Resting FVR (mmHg ml⁻¹ min 100 ml)</td>
<td>14·3±2·0</td>
<td>16·4±2·1</td>
<td>&lt;0·01</td>
</tr>
<tr>
<td>Resting Pa,O₂ (mmHg)</td>
<td>90·4±1·7</td>
<td>586·4±9·8</td>
<td>&lt;0·001</td>
</tr>
<tr>
<td>Resting Pa,CO₂ (mmHg)</td>
<td>37·8±0·8</td>
<td>36·9±1·3</td>
<td>NS</td>
</tr>
<tr>
<td>Resting Pv,O₂ (mmHg)</td>
<td>40·4±3·0</td>
<td>61·9±4·8</td>
<td>&lt;0·01</td>
</tr>
<tr>
<td>Ischaemic MABP (mmHg)</td>
<td>47·2±5·6</td>
<td>49·8±5·6</td>
<td>&lt;0·05</td>
</tr>
<tr>
<td>Ischaemic Pv,O₂ (mmHg)</td>
<td>23·0±2·0</td>
<td>38·9±3·3</td>
<td>&lt;0·01</td>
</tr>
<tr>
<td>Ischaemic Pv,CO₂ (mmHg)</td>
<td>45·8±1·6</td>
<td>44·7±1·5</td>
<td>NS</td>
</tr>
<tr>
<td>Maximum post-ischaemic FBF (ml min⁻¹ 100 ml⁻¹)</td>
<td>19·7±2·1</td>
<td>18·2±2·4</td>
<td>NS</td>
</tr>
<tr>
<td>Minimum post-ischaemic FVR (mmHg ml⁻¹ min 100 ml)</td>
<td>4·5±0·6</td>
<td>5·2±0·7</td>
<td>NS</td>
</tr>
</tbody>
</table>

All values are mean ± SEM. P refers to comparison of difference between room air and 100% oxygen values to zero by means of t-test. NS = not significant. Abbreviations as in Table 1.

In five subjects the responses to ischaemia while the subjects breathed room air and during 100% oxygen breathing were compared. During ischaemia with oxygen breathing, venous blood oxygen tension was not different from its value seen during air breathing with free circulation by more than 1·9 mmHg in four subjects, but it was lower than the latter by 5·5 mmHg in the fifth subject. In spite of the reasonably satisfactory maintenance of venous blood PO₂ to a level seen during room air breathing with the circulation free, the response to ischaemia was not modified significantly (Table 7 and Fig. 8).

To simulate the effects of ischaemia more closely, the vasodilator response to ischaemia was compared to that produced by a combination of hypoxia and hypercapnia in six subjects. The decrease in vascular resistance caused by combined hypoxia and hypercapnia averaged 64% of that produced by ischaemia (Table 8, Fig. 9).
### TABLE 8. Comparison of vasodilator response to ischaemia and that produced by combined hypoxia and hypercapnia

<table>
<thead>
<tr>
<th></th>
<th>Ischaemia</th>
<th>Combined hypoxia and hypercapnia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting MABP (mmHg)</td>
<td>87.2 ± 4.1</td>
<td>88.0 ± 4.3</td>
</tr>
<tr>
<td>Resting FBF (ml min⁻¹ 100 ml⁻¹)</td>
<td>7.0 ± 0.5</td>
<td>7.6 ± 0.5</td>
</tr>
<tr>
<td>Resting FVR (mmHg ml⁻¹ min 100 ml)</td>
<td>12.9 ± 1.3</td>
<td>11.9 ± 1.0</td>
</tr>
<tr>
<td>Resting ( P_{v,O_2} ) (mmHg)</td>
<td>49.5 ± 4.8</td>
<td>49.3 ± 4.7</td>
</tr>
<tr>
<td>Resting ( P_{v,CO_2} ) (mmHg)</td>
<td>40.6 ± 0.6</td>
<td>39.8 ± 0.8</td>
</tr>
<tr>
<td>MABP (during ischaemia or combined hypoxia and hypercapnia) (mmHg)</td>
<td>56.8 ± 2.6</td>
<td>97.8 ± 5.3</td>
</tr>
<tr>
<td>( P_{v,O_2} ) (during ischaemia or combined hypoxia and hypercapnia) (mmHg)</td>
<td>33.9 ± 1.6</td>
<td>37.6 ± 2.2</td>
</tr>
<tr>
<td>( P_{v,CO_2} ) (during ischaemia or combined hypoxia and hypercapnia) (mmHg)</td>
<td>43.5 ± 0.3</td>
<td>42.9 ± 0.5</td>
</tr>
<tr>
<td>FBF (maximum post-ischaemic or during combined hypoxia and hypercapnia) (ml min⁻¹ 100 ml⁻¹)</td>
<td>18.4 ± 2.0</td>
<td>14.8 ± 1.0</td>
</tr>
<tr>
<td>FVR (minimum post-ischaemic or during combined hypoxia and hypercapnia) (mmHg ml⁻¹ min 100 ml)</td>
<td>4.9 ± 0.4</td>
<td>6.8 ± 0.6</td>
</tr>
</tbody>
</table>

All values are mean ± SEM. Abbreviations as in Table 1.

![Graph](image_url)

**FIG. 9.** Comparison of the response to ischaemia produced by brachial arterial occlusion (A) with that produced by combined hypoxia and hypercapnia induced by breathing gas with high concentration of CO₂ and low concentration of oxygen (B). Abbreviations are the same as in Fig. 6. Breathing of low concentrations of oxygen with high concentrations of CO₂ was begun at the seventh min in panel B.
DISCUSSION

The vascular bed studied and the conditions of the present experiments appeared to have been well suited for demonstrating the full extent of the local vasodilator effect of hypoxia. The vascular bed of the human forearm exhibits vigorous autoregulatory responses. This is true for both the intact and for the phenoxybenzamine and propranolol treated forearm, although in the latter the response to ischaemia was reduced by about one-third probably because the vessels were more dilated. Possible depression of vasodilator responses by surgery or anaesthesia were not factors in this investigation. The use of adrenergic blocking agents inhibited vasodilator and vasoconstrictor effects of catecholamines and the vasoconstrictor effect of increased activity of sympathetic nerves. As shown above, the associated hypocapnia did not influence the results. This might seem paradoxical on superficial examination. More careful consideration, however, shows that this is not the case. While hypocapnia induced by sudden vigorous hyperventilation is associated with forearm muscle vasodilatation, this response is transient and does not occur at all when arterial blood $PCO_2$ is reduced to a low level in a more gradual manner, as is the case for hypocapnia associated with hypoxia (Richardson et al., 1965).

The present findings confirmed previous observations that hypoxia is vasodilator in the human forearm. The data did not distinguish whether the response occurred in forearm muscle or forearm skin or in both. As noted above, the magnitude of the vasodilator response to hypoxia is of importance with respect to the role of hypoxia in the local regulation of blood flow. In the present investigation significant vasodilator responses were observed when arterial blood $PO_2$ decreased below 45 mmHg, or when venous blood $PO_2$ decreased below 35-40 mmHg. Even at the lowest levels of oxygen tension obtained in the present investigation, the vasodilator response was of relatively small magnitude. These observations are consistent with the view expressed by Daugherty et al. (1967) that hypoxia cannot be the sole cause of autoregulatory phenomena in the vascular bed of the extremities. Its role may be more profitably considered under those physiological circumstances where decrease in oxygen tension in the environment of the high resistance vessels is pronounced.

As shown above, $CO_2$ breathing under the circumstances of our experiments is capable of producing forearm vasodilatation of at least equal magnitude to that produced by ischaemia. We believe that a similar interpretation may be reasonably made of the experiments of Fairchild et al. (1966), namely that, under the conditions of their experiments, the absence of oxygen is capable of producing vasodilatation which is equal in magnitude to that produced by prolonged ischaemia. It would be reasonable to expect that the same result might be achieved with any strong vasodilator agent. The absence of flow overshoot in their experiments when the circulation was restored might have been due to maximal dilatation of the vascular bed, as might reasonably have been expected after a 10-min period of ischaemia.

The increase in the maximal flow following ischaemia when $CO_2$ was breathed was undoubtedly in part related to the higher arterial blood pressure. This could not, however, have been the only reason for the increase in flow because the latter was far in excess of what would have been accounted for by the modest increase in blood pressure. Blair, Glover, McArdle & Roddie (1960) found that a period of ischaemia following $CO_2$ breathing was associated with reactive hyperaemia which was of greater magnitude and duration than when ischaemia followed a period of air breathing. They showed that this was related to the increase in $CO_2$ tension in the ischaemic tissues. It is likely that a similar mechanism accounted for the increase in maximal flow following ischaemia during $CO_2$ breathing in the present study.
The present findings suggest that a major portion of the decrease in vascular resistance produced by ischaemia can be accounted for by local hypercapnia. In contrast, the results suggest that the contribution of local hypoxia to the response to ischaemia is quantitatively much less important. These results are, however, pertinent to incomplete arrest of the circulation where the decrease in venous blood $PO_2$, and by inference in tissue $PO_2$, is not very marked. The possibility that the contribution of local hypoxia might be greater than inferred from the present study under conditions of more severe ischaemia cannot be excluded. Our findings with regard to hypoxia are in agreement with those of other investigators who showed that in the anaesthetized dog autoregulation of skeletal muscle blood vessels was present in the absence of oxygen or in the presence of very high venous blood oxygen tension (Daugherty et al., 1967; Bond, Blackard & Taxis, 1969).

In the present study, the venous blood gas tensions were used as reasonable approximations of the mean tissue gas tensions. It is recognized, however, that $PO_2$ and $PCO_2$ are likely to be different at different sites within the tissues. It would have been more appropriate to relate the changes in vascular resistance during and after ischaemia and during hypoxia or hypercapnia to the gas tensions at the sites where $CO_2$ and oxygen act to produce the changes in vascular resistance. The available evidence suggests that $CO_2$ produced vasodilatation by direct action on vascular smooth muscle, probably as a result of decrease in intracellular pH (Kontos, Richardson & Patterson, 1968). Hence, it would have been more appropriate to measure $PCO_2$ in the extracellular fluid in the vicinity of vascular smooth muscle or the intracellular $PCO_2$ of $pH$ of these cells. There are two views concerning the mechanism of action of hypoxia. According to the first, hypoxia acts directly on the smooth muscle of the high resistance vessels (Guyton et al., 1964). According to the second, hypoxia induces release of vasodilator metabolites from the tissues which in turn act on the vascular smooth muscle producing relaxation (Berne, 1964). If the first hypothesis is correct, the appropriate sites for $PO_2$ measurement would have been either the extracellular fluid in the vicinity of the vascular smooth muscle or the intracellular $PO_2$ of the smooth muscle cells. If the second view is correct, the intracellular $PO_2$ of the skeletal muscle cells would have been more appropriate. Reliable measurements of the gas tensions at these sites under the conditions of the present experiments are not available, and it is not possible to estimate errors that might have arisen as a result of the absence of such measurements.

As shown above, during ischaemia the vessels of the forearm were perfused with collateral flow of blood having the same gas tensions as normal arterial blood. Hence, under these conditions, the only way that hypoxia or hypercapnia would have affected directly the high resistance precapillary vessels is through a change in the gas tensions at the external surface of their wall. In contrast, during hypoxia or hypercapnia, induced by breathing the appropriate gas mixtures, the gas tensions in the arterial blood and quite probably in the tissues were changed. Hence, the precapillary high resistance vessels under these conditions had both the internal and external surfaces of their wall exposed to altered gas tensions. It is therefore possible that, for the same venous blood oxygen or $CO_2$ tension, the degree of hypoxia or hypercapnia of the vascular wall of the high resistance vessel might have been more severe during breathing of low oxygen or high $CO_2$-containing gas mixtures than during ischaemia. This would have resulted in overestimation of the contribution of local hypercapnia and local hypoxia to the response to ischaemia.

Although the present findings do not exclude the possibility of mutual potentiation of the
vascular effects of hypoxia and hypercapnia, they suggest that if such an effect does indeed occur its magnitude must be relatively small. This is based on the fact that the vasodilator response to combined hypoxia and hypercapnia was not substantially greater than the additive effects of hypoxia alone and hypercapnia alone.

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REFERENCES


