Effects of Anoxia on the Vascular Resistance of the Dog's Hind Limb

By Jerzy Litwin, M.D., Abdul Habib Dil, M.D., and Domingo M. Aviado, M.D.

Anoxia is known to induce a biphasic effect on blood flow to the extremities. In anesthetized dogs, there is a decrease in blood flow to the leg during anoxia but this is immediately followed by an increase during reoxygenation. Since thermoelectric methods have been used for recording blood flow, it has not been possible to express the vascular responses quantitatively to the same extent as the effects of anoxia on cardiac output and pulmonary vascular resistance.

There is general agreement that the initial reduction in blood flow to the extremities is due to neurogenic vasoconstriction, but the role of chemoreceptors in the carotid and aortic bodies lacks direct proof. The increase in blood flow to the extremities during reoxygenation is due to actual vasodilatation, but the mechanism has been claimed to be either nervous or local in nature. The experiments that are reported below are attempts to fill these existing gaps in information. The role of nervous mechanisms is explored by surgical denervation and by a new type of sympathetic blocking drug, bretylium bromide. This drug has been shown to block selectively the sympathetic nerves but spare the actions of catecholamines.

Methods

The experiments were performed on 47 mongrel dogs, weighing 10 to 24.5 Kg, which were anesthetized with morphine sulfate (2 mg./Kg. s.c.) and chloralose (70 mg./Kg. i.v.). In all experiments, the chest was opened in the right fifth intercostal space and the lungs were ventilated by means of a Starling Ideal Pump. A mixture of 5 per cent oxygen in nitrogen was administered through this pump. Mannuronate (10 mg./Kg.) or heparin (200 to 400 I.U./Kg.) was injected intravenously as an anticoagulant. A Sanborn PolyViso was used to record the following: (a) aortic blood pressure by a Statham transducer from a catheter inserted via the right femoral artery; (b) inferior vena cava pressure by a second Statham transducer from a catheter inserted in the corresponding femoral vein; and (c) special measurements on the left hind leg, which will be described under each of the 3 types of experiments.

Measurement of Blood Flow (15 Dogs)
The left iliac artery was ligated and cannulated peripherally. One common carotid artery was cannulated and this was connected by rubber tubing to the iliac arterial cannula. Blood flowing into the iliac artery was recorded by a Shipley-Wilson rotameter.

Perfusion of Hind Leg (20 Dogs)
In a second group of dogs, a Sigmamotor pump was inserted between the common carotid and the left iliac artery to supply blood at a constant rate of flow. Perfusion pressure into the iliac artery (left leg) was recorded by means of a Statham transducer. In some of these dogs, pressure in the right femoral artery distal to the ligature was measured by means of another Statham transducer. This offered an opportunity to compare changes in vascular resistance of the perfused left leg with corresponding changes in pressure in the ligated vessels of the right leg. The latter has been used by Nolf to represent changes in vascular resistance.

Cross-Circulation Experiments (3 Experimental and 3 Donor Dogs)
In a third group of dogs, the perfusion of the hind limb was completed by using a donor dog. The left iliac artery of the experimental dog was supplied with blood from one common carotid of the donor dog by means of a pump. Blood from the corresponding iliac vein was returned to the
Effects of inhalation of 5 per cent oxygen on aortic blood pressure, femoral venous pressure and iliac arterial blood flow. The latter remained unchanged in spite of a rise in aortic blood pressure, indicating vasoconstriction. During reoxygenation, there is an increase in blood flow.

Results

Anoxia on Limb Blood Flow

The first group of experiments in 15 dogs consisted of measurements of aortic blood pressure and flow in the iliac artery. This allowed a calculation of vascular resistance by dividing pressure by blood flow. The inhalation of 5 per cent oxygen for 1 to 3 minutes induced 2 types of responses: during anoxia and during reoxygenation (table 1 and fig. 1).

During Anoxia

The aortic blood pressure increased progressively during the inhalation of 5 per cent oxygen (mean +19 per cent). In spite of this consistent rise, blood flow to the extremities increased to a lesser extent, remained unchanged or even reduced (mean —5; range —27 to +13 per cent). Since femoral venous pressure remained unchanged, vascular resistance in the hind limb was calculated by dividing aortic blood pressure by flow and the results showed an increase during anoxia (mean +23 per cent).

During Reoxygenation

The responses immediately following cessation of anoxia were more consistent than those during anoxia. Blood flow increased by a mean value of +63 per cent (range +9 to +114). The aortic blood pressure was at a level lower than that during anoxia, but was either higher or lower than the control level (—27 to +11 per cent). Vascular resistance was consistently lower than the control level (mean —40 per cent; range —16 to —62 per cent).

It is clear from the above results that 2 sets of responses require investigation: (a) an increase in vascular resistance during anoxia; and (b) a decrease during reoxygenation. The former was not a constant feature in the above experiments, but the next group of experiments showed a more uniform response.

Anoxia on Perfused Limb

The addition of a perfusion pump to keep blood flow to the iliac artery at a constant level gave the following results in 20 dogs (table 1): There was a rise in perfusion pressure ranging from 3 to 47 per cent, with a mean response of +12 per cent during the inhalation of 5 per cent oxygen. On the readmission of air, there was a complete re-
### Table 1

**Summary of Responses to Anoxia and During Reoxygenation**

<table>
<thead>
<tr>
<th>Type of experiment</th>
<th>Responses to Anoxia</th>
<th>During Reoxygenation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of observations</td>
<td>Mean ± S.E. (range)</td>
</tr>
<tr>
<td>Perfusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not perfused</td>
<td>15</td>
<td>132±9 (55 to 186)</td>
</tr>
<tr>
<td>Blood flow measured</td>
<td>17</td>
<td>118±5.8 (75 to 170)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Not measured</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Not measured</td>
</tr>
<tr>
<td>Perfused limb</td>
<td>20</td>
<td>86±8.8 (60 to 110)</td>
</tr>
<tr>
<td>(1 dog)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Perfused limb</td>
<td>3</td>
<td>50±4 (26 to 75)</td>
</tr>
<tr>
<td>(2 dogs)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2**

**Responses to Anoxia and During Reoxygenation: Effect of Denervation and Drugs**

<table>
<thead>
<tr>
<th>Line no.</th>
<th>Type of experiment</th>
<th>No. of dogs</th>
<th>Mean</th>
<th>% A 5% oxygen</th>
<th>S.E.*</th>
<th>Range</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>3</td>
<td>4</td>
<td>+10</td>
<td>±2.95</td>
<td>+6 to +16</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>2</td>
<td>Adrenergic</td>
<td>3</td>
<td>8</td>
<td>+2.5</td>
<td>±1.9</td>
<td>-6 to +11</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>3</td>
<td>Chloroethylamine</td>
<td>3</td>
<td>3</td>
<td>+12</td>
<td>±5.8</td>
<td>+5 to +18</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>4</td>
<td>Atropine</td>
<td>3</td>
<td>3</td>
<td>+3.5</td>
<td>±3.5</td>
<td>-7 to 0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>5</td>
<td>Hexamethonium</td>
<td>3</td>
<td>3</td>
<td>-17</td>
<td>±1.7</td>
<td>-8 to -27</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>6</td>
<td>Transection</td>
<td>3</td>
<td>3</td>
<td>-12</td>
<td>±12</td>
<td>-15 to -10</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*P = Standard error of the mean.

*S.E. = Standard error of the mean.
versal with a fall in perfusion pressure of 6 to 59, a mean of —28 per cent from the preanoxic control level. Since rate of flow during both periods was unchanged from the control period, these changes in perfusion pressure would correspond to the alterations in vascular resistance. The perfusion technic showed consistent changes in vascular resistance and was therefore used to study the mechanisms for the increase and decrease in vascular resistance, during and following anoxia.

Responses of Perfused Limb and Limb with Vessels Ligated

Blood pressure at the peripheral end of the ligated femoral artery (right side) behaved in a manner similar to the pressure in the perfused limb (left side). During anoxia, pressures of both legs rose. Thirteen observations are summarized in figure 2, showing that the per cent increases in pressure in the distal artery were more intense than the corresponding increases in perfusion pressure of the other leg. Nolf’s method of using pressure in the distal artery to represent vascular resistance is valid in this group of experiments, but it overrates the extent of vasoconstriction as compared to that measured by perfusion. During reoxygenation, the fall in perfusion pressure was accompanied by a fall in distal arterial pressure in all 13 observations, except in 1 instance in which there was a rise. The decrease in vascular resistance suggested by the fall in distal arterial pressure is either less intense or more intense than the decrease measured by perfusion. These observations serve to compare 2 methods of measuring vascular resistance, but only the perfusion method was applied for investigation of mechanism for vasoconstriction and vasodilatation.

Denervation of the Hind Limb

The immediate appearance of vasoconstriction (during anoxia) and vasodilatation (during reoxygenation) suggested that both were mediated by a nervous mechanism. A response from 1 dog, typical of 3 others, is depicted in figure 3, in which the control response to anoxia consisted of a rise in aortic blood pressure, a rise in perfusion pressure of the hind limb, and then a fall of the latter during reoxygenation. All nerves to the perfused extremities, as well as the nerve fibers adjoining the iliac artery, were sectioned and inhalation of 5 per cent oxygen was repeated. The rise in aortic blood pressure was still present, the vasoconstriction of the leg was either reduced or reversed, but the vasodilatation during reoxygenation was reduced in intensity (see table 2, line 1 for summary and statistical
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Although the iliac vein of the perfused limb was intact, venous pressure remained unchanged, so that the observed changes in the perfused artery reflect vascular resistance for the entire limb. The remaining responses after denervation of the extremities are mediated through 2 mechanisms which may have some local action on the perfused vessels: (a) catecholamines, liberated from the adrenal medulla during anoxia; and (b) reduction in oxygen content of blood reaching the perfused leg.

**Adrenalectomy and Bretylium**

The next obvious step was to add adrenalectomy to the above dogs with denervated extremities. This was not accomplished successfully because the surgical trauma involved in denervation, subsequent adrenalectomy and leg perfusion resulted in shock. Four dogs were initially adrenalectomized and the responses to anoxia were essentially similar to those prior to removal of the adrenals (fig. 4). Denervation of the limb was accomplished by intravenous injection of bretylium bromide, a drug which has been shown to block sympathetic vasoconstrictor nerves. The response to anoxia was characterized by a lack of rise in aortic blood pressure and vasoconstriction of the perfused limb. The postanoxic vasodilatation still persisted and was as intense as that encountered in the intact animal (table 2, line 2). These results re-emphasize that vasoconstriction and vasodilatation are not mediated by one common mechanism and the next group of procedures will offer additional evidence.

**Denervation of Carotid and Aortic Bodies**

The role of chemoreceptors in the carotid and aortic bodies was examined in 3 dogs and a representative record is depicted in figure 5. After denervation, the inhalation of 5 per cent oxygen now caused a fall in aortic blood pressure and a fall in perfusion pressure, indicating vasodilatation of the perfused limb. After cessation of anoxia, there was a further fall in perfusion pressure or vasodilatation (table 2, line 3).

**Atropine**

So far, the postanoxic vasodilatation was unaffected either by bretylium or by chemoreceptor denervation, although both procedures eliminated the anoxic vasoconstriction. The injection of atropine sulfate (1 to 2 mg./Kg. i.v.) caused an opposite effect which was as follows: the vasoconstrictor response of the perfused leg was unaffected, but the postanoxic vasodilatation was reduced to about half the control response (table 2, line 4).

**Hexamethonium**

This ganglion blocking agent was administered intravenously in 4 dogs in doses of 1 to 5 mg./Kg. (table 2, line 5). There was a definite reduction in all the responses during and immediately following anoxia, but they were not completely eliminated.

** Transection of the Spinal Cord**

The only successful way to eliminate completely the postanoxic vasodilatation was to section the spinal cord at the level of C2 to C3. This was performed in 2 dogs in which both the vasoconstrictor (during anoxia) and vasodilator responses (during reoxygenation)
Responses to anoxia before and after chemoreceptor denervation. The anoxic increase in perfusion pressure (vasoconstriction) disappears after denervation, but the postanoxic decrease (vasodilatation) persists.

Cross-Circulation Experiments

The perfusion experiments described in the above paragraphs consisted of a single animal in which the iliac artery was supplied with blood from the same animal. This meant that the neurogenic vasoconstriction during anoxia and vasodilatation during reoxygenation occurred while the oxygen content of the perfusate was altered correspondingly. The oxygen content of the blood was kept constant by means of cross-circulation experiments in which a donor dog was used to supply blood for the hind limb of the experimental dog. The administration of 5 per cent oxygen to the latter (while the former was ventilated with room air), caused the usual vasoconstriction, followed by vasodilatation on readmission of air (table 1 and fig. 6). The limb was then denervated and like the single dog perfusion experiments, the intensity of both responses was significantly reduced.

Discussion

Increased Vascular Resistance during Anoxia

Vasoconstriction of the extremities is the most uniform response encountered during anoxia in the 3 groups of experiments. Another glance at table 1 indicates that the increase in vascular resistance in the first group of dogs (+23 per cent) is considerably higher than that in the second group (+12 per cent). This difference is probably related to the technic of measuring vascular resistance: the first group of dogs utilized a rotameter in the iliac artery and vascular resistance was measured by the ratio of pressure and flow, both of which were altered during anoxia. It has been shown by Green et al. that calculation of resistance, when both pressure and flow to the extremities vary, lead to erroneous answers because of passive changes in resistance when either flow or pressure alone is varied. On the other hand, the second group of dogs had their extremities perfused at a constant blood flow, so that the responses in resistance are more representative of the effects of anoxia on the vascular tone of the limb vessels.

The second and third groups of dogs were investigated for vascular resistance by perfusion method, yet the average increase in resistance for the latter was higher (+35 per cent) than the former (+12 per cent). The difference between the 2 groups lies in the constant composition of blood perfusing the leg in the third group by means of cross-circulation experiments, so that the increase in resistance of +35 per cent represents the effect of the systemic anoxemia on the perfused leg by nervous pathways. On the other hand, the second group of dogs with an increase of 12 per cent during anoxia occurred during a combined action of systemic anoxia (increased resistance) minus the local influence of anoxemia on the leg vessels as well as the local effects of liberated catecholamines. These 2 types of local actions were not segregated, but their combined action is that of dilatation to reduce the intensity of vasoconstriction (from +35 per cent of pure neurogenic influences) to +12 per cent.

Mechanisms for Vasoconstriction

Three procedures were effective in eliminating the vasoconstrictor response of the leg vessels during inhalation of 5 per cent oxy-
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They are as follows: (a) denervation of chemoreceptors in the carotid and aortic bodies; (b) transection of the spinal cord; and (c) adrenalectomy, combined with intravenous injection of bretylium, to block the sympathetic nerves. These results identify the chemoreceptors as the primary source of excitation by anoxemia. The reflex excitation of the vasoconstrictor center in the medulla finally reaches the blood vessels by way of the sympathetic nerves, and also by liberation of catecholamines by adrenal medulla. These results essentially confirm those of Bernthal et al.1 and of Rein et al.3

**Decreased Vascular Resistance during Reoxygenation**

The 3 groups of dogs show different intensity of vasodilator responses during reoxygenation. The significance of these differences is probably similar to those described for the vasoconstrictor responses and are as follows:

The third group of dogs consisting of cross-circulation experiments represents the dilator response (mean of −21 per cent) exclusive of local effects of alteration in blood gases. The second group of dogs in which the leg was exposed to changes in blood composition showed a vasodilator response slightly more intense (−28 per cent) but the differences between these 2 groups are not as severe as that encountered for the vasoconstrictor response during anoxia. Although changes in oxygen content of blood influence the intensity of vasoconstriction, this does not appear to be as important as in the vasodilation during reoxygenation.

**Mechanism for Vasodilatation**

The explanation for postanoxic vasodilation is not completely known. The experiments reported above show that it is neurogenic in origin because either denervation of the limb or transection of the spinal cord reduced significantly the intensity of the postanoxic vasodilatation. Bernthal10 showed that anoxemia of the perfused carotid bodies can elicit vasodilatation following vasoconstriction, but the above results show that this vasodilatation can be elicited even after denervation of the chemoreceptors. This vasodilation that is induced by neurogenic pathways can be elicited in 2 ways, i.e., by chemoreceptors or by medullary stimulation.

One shortcoming of the above experiments is that the procedures that eliminated the postanoxic vasodilation might have induced maximal vasodilation prior to induction of anoxia. This was disproved by showing that the perfused legs after denervation could still dilate to local injections of either acetylcholine or histamine. The theory that postanoxic vasodilatation is exclusively a local mechanism, as proposed by Rein et al.3 and by Schneider,3 is not supported by the above experiments.

The demonstration that postanoxic vasodilatation is neurogenic in origin raises a number of questions that require additional investigation. The nervous pathways should be segregated between the sympathetic and so-
matic nerves, as well as between the dorsal root and ventral root, as described by others.\textsuperscript{11, 12} Pharmacologically, the vasodilation can be reduced by either ganglion-blocking drugs, or by atropine, but not by bretylium. These observations imply that the nervous pathways are susceptible to drugs that block cholinergic transmitters. The resistance of these nerves to bretylium suggests that these fibers are unlike sympathetic fibers that induce vasoconstriction and cardioacceleration.\textsuperscript{7}

The most puzzling feature of these dilator mechanisms is their manifestation only during reoxygenation. It is understandable that during anoxia, this neurogenic dilatation is masked by reflex vasoconstriction from chemoreceptors. After chemoreceptor denervation, one should expect neurogenic dilatation, but presumably this is masked by stimulation of medullary vasoconstrictor centers. Evidence against this possibility\textsuperscript{13} is as strong as evidence in favor of it,\textsuperscript{14} so that a complete explanation must await additional investigation.

One aspect of the vasodilatation which deserves some serious attention is its relationship to the vasodilatation reported by Beck.\textsuperscript{15-17} The blood vessels of the dog's hind limb are dilated reflexly by the systemic administration of either epinephrine or levarterenol. This dilator response is blocked by antihistaminic drugs. A similar vasodilatation following asphyxiation can be blocked by antihistaminic drugs (personal communication from L. Beck). It is therefore probable that the postanoxic vasodilatation reported in the above experiments is partly induced by neurogenic mechanisms releasing histamine and blocked by antihistaminic drugs.

If the vasoconstrictor center is stimulated directly during anoxia, the vasodilatation during reoxygenation may arise from a recession of activity of this same center below its normal level of discharge, as suggested by Bernthal and Schwind.\textsuperscript{18} On the other hand, the vasodilation could arise from a different center mediating vasodilation. Such a dilator response may last longer than stimulation of the vasoconstrictor center, so that reoxygenation would mean disappearance of activity of the latter but persistence of the former.

**Summary**

In anesthetized dogs, the inhalation of 5 per cent oxygen caused vasoconstriction of perfused hind limb but vasodilatation occurred during reoxygenation. The vasoconstriction during anoxia amounted to a mean increase of vascular resistance of +12 per cent if blood from the same anoxic dog was used for perfusion, and to +35 per cent if blood from a nonanoxic donor was used. This vasoconstrictor response was almost entirely the outcome of anoxic stimualtion of chemoreceptors in the carotid and aortic bodies. The vasodilatation during reoxygenation persisted after chemoreceptor denervation, but was eliminated by either spinal cord transection or denervation of the limb. The nervous pathways responsible for this postanoxic vasodilatation were resistant to bretylium but were partially blocked by either atropine or hexamethonium.

**Summario in Interlingua**

In anesthesiate canes, le inhalation de 5 pro cento de oxygeno causava vasoconstriction del perfusionate extremitate posterior, sed vasodilatation occurreva durante le reoxygenation. Le vasoconstrictione durante le anoxia se exprimeva in un augmento medio del resistentia vascular de 12 pro cento quando sanguine ab le mesme can esseva utase in le perfusion e do 35 pro cento quando le sanguine del perfusion veniva ab un donator non-anoxic. Iste responsa vasoconstrictori esceva quasi totalmente le effetto de stimulation anoxicemie de chemoreceptores in le corporis carotidici e aortici. Le vasodilatation durante le reoxygenation persisteva post le disnervation del chimorcceptores, sed illo esseva eliminate per le transsection del medulla spinal e disnervation del extremitate. Le circuitos nervose quo es responsable pro iste vasodilatation postanoxic esseva refractori a bretylium, sed illos poteva esser blocate per atropina e etiam per hexamethonium.

**References**


2. —: Some observations upon changes in volume


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